ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES
FISCAL YEAR 1979

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service   National Institutes of Health

Mailing List 9/24/81

Report, 1979.

United States, NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

FY 1979 ANNUAL REPORT

October 1, 1978 through September 30, 1979

RA
1231
U56
1978-79

CONTENTS

Page

INDEXES
- Extramural Research Highlights. v
- Individual Project Reports. vii
- Research Contract Narratives. xxi

OFFICE OF THE DIRECTOR
- Summary Statement xxvi

EXTRAMURAL PROGRAM
- Office of the Associate Director for Extramural Program, Summary Statement. 2
- Training Programs: Research Manpower Development Section . . . 4
- Research Highlights: Environmental Health Sciences (EHS) Center Program. 10
- Research Highlights: Regular Research Grants Program 43

GENETICS
- Office of the Associate Director for Genetics, Summary Statement 72

HEALTH HAZARD ASSESSMENT
- Office of Health Hazard Assessment, Summary Statement 78
- Individual Project Reports. 82
- Research Contract Narratives. 102

INTERAGENCY PROGRAMS
- Office of the Associate Director for Interagency Programs, Summary Statement. 108

PROGRAM PLANNING AND EVALUATION
- Office of Program Planning and Evaluation, Summary Statement. . 112

INTRAMURAL PROGRAM
- Office of the Scientific Director, Summary Statement. 117
- BIOMETRY BRANCH
  - Summary Statement. 122
  - Individual Project Reports 127
  - Research Contract Narratives 158
- LABORATORY OF ANIMAL GENETICS
  - Summary Statement. 160
  - Individual Project Reports 164
  - Research Contract Narratives 198
- LABORATORY OF BEHAVIORAL AND NEUROLOGICAL TOXICOLOGY
  - Summary Statement. 204
  - Individual Project Reports 208
  - Research Contract Narratives 252
- LABORATORY OF BIOCHEMICAL GENETICS
  - Summary Statement. 256
  - Individual Project Reports 259
  - Research Contract Narratives 275

Page
LABORATORY OF ENVIRONMENTAL BIOPHYSICS
Summary Statement. 286
Individual Project Reports 293
Research Contract Narratives 398
LABORATORY OF ENVIRONMENTAL TOXICOLOGY
Summary Statement. 412
Individual Project Reports 428
Research Contract Narratives 486
LABORATORY OF MOLECULAR GENETICS
Summary Statement. 496
Individual Project Reports 499
Research Contract Narratives 535
LABORATORY OF ORGAN FUNCTION AND TOXICOLOGY
Summary Statement. 541
LABORATORY OF PHARMACOKINETICS
Summary Statement. 548
Individual Project Reports 551
Research Contract Narratives 567
LABORATORY OF PHARMACOLOGY
Summary Statement. 576
Individual Project Reports 579
LABORATORY OF PULMONARY FUNCTION AND TOXICOLOGY
Summary Statement. 618
Individual Project Reports 623
Research Contract Narratives 679

RESEARCH RESOURCES PROGRAM
Office of the Director for Research Resources Program, Summary Statement. 683
COMPARATIVE MEDICINE BRANCH
Summary Statement. 692
Individual Project Reports 696
ENVIRONMENTAL BIOLOGY BRANCH
Summary Statement. 708
Individual Project Reports 714
Research Contract Narratives 763
ENVIRONMENTAL CHEMISTRY
Summary Statement. 774
Individual Project Reports 781
Research Contract Narratives 835
RESEARCH SERVICES BRANCH
Summary Statement. 840

INDEX TO EXTRAMURAL RESEARCH HIGHLIGHTS

ENVIRONMENTAL HEALTH SCIENCES (EHS) CENTERS PROGRAM

Centers Page

Mount Sinai School of Medicine. 10
  Human Health Effects of Long-term, Low-level Exposure to Environmental Agents. 10
  Multiple Factor Interaction of Environmental Agents 11

New York University Medical Center. 13
  Research in Toxicology. 13
  Chemical Carcinogenesis 14
  Research in Radiation Dosimetry and Carcinogenesis. 17
  Research in Respiratory Disease in Aerosol Physiology 17

Vanderbilt University 19

Harvard University. 25
  Epidemiological Studies 26

Oregon State University 27

University of Rochester 30

University of Cincinnati. 35

Massachusetts Institute of Technology 40

REGULAR RESEARCH GRANTS PROGRAM

Mutagenesis and Carcinogenesis Test Development 43

Air Pollutants. 46

Metals. 49

Metallothionein 50

Physical Factors. 57
  Noise 57
  Microwave Irradiation 58
  High Voltage Transmission Lines 58
  Photosensitivity. 59

Natural Toxins. 59

Subject Page

Biomechanisms. 62

Organic Chemicals. 67

INDEX TO INDIVIDUAL PROJECT REPORTS

OFFICE OF HEALTH HAZARD ASSESSMENT

Project Number	Title	Page
Z01 ES 20001-07 OHHA	Variations in the Response of Rats to Porphyrogenic Chemicals	82
Z01 ES 20002-07 OHHA	Technology Forecasting and Technology Assessment	85
Z01 ES 20003-06 OHHA	Preventive Surveillance of Environmental Chemicals for Toxic Potential	88
Z01 ES 20004-05 OHHA	Surveillance of Potential Environmental Health Hazards	92
Z01 ES 20007-02 OHHA	Identification and Evaluation of Environmental Health Hazards: Chemicals and Chemical Carcinogens	95
Z01 ES 20008-02 OHHA	The Marsupial Neonate as a Model for the Identification and Evaluation of Environmental Toxicants	97
Z01 ES 20009-01 OHHA	Identification of Potential Environmental Health Hazards	100

BIOMETRY BRANCH

Project Number	Title	Page
Z01 ES 40002-09 BB	Statistical Methodology Development	127
Z01 ES 40004-02 BB	Statistical Methods in Epidemiology	130
Z01 ES 40005-02 BB	Statistical Analysis of Mutagenesis Testing Data	132
Z01 ES 41001-05 BB	Risk Assessment for Environmental Carcinogens	134
Z01 ES 42002-07 BB	Pharmacokinetic Modeling and Methodology Development	137
Z01 ES 43002-03 BB	Environmental Contaminants of Infant Foodstuffs-The Breast Milk and Formula Project	142
Z01 ES 43003-01 BB	Studies in Pediatric Lead Exposure	144
Z01 ES 43004-01 BB	Skin Cancer and Trace Elements	146

Project Number	Title	Page
Z01 ES 43005-01 BB	Reproductive Experience of Drycleaning Workers and Their Conjugal/Marital Partners	149
Z01 ES 43006-01 BB	Genetic EFfects, Reproductive Outcomes and Birth Defects and Occupational Exposure to Plutonium	152
Z01 ES 44002-04 BB	Mathematical Population Genetics	154
Z01 ES 44003-02 BB	Fetal Wastage and Chemical Exposure	156
	LABORATORY OF ANIMAL GENETICS	
Z01 ES 60042-04 LAG	The Study of Recessive Lethal Mutations Induced in Mice by Chemical Mutagens	164
Z01 ES 60057-03 LAG	Molecular Analyses of Mitochondrial DNA's of Different Species of Drosophila	166
Z01 ES 60058-03 LAG	Amount and Effects of Null Allozymic Variation in Natural Populations	168
Z01 ES 60059-03 LAG	Effects of Chromosomal Aberrations on Embryonic Development	170
Z01 ES 60062-03 LAG	Molecular Characterization of Isozymes and Mutant Enzymes in Mammals	172
Z01 ES 60063-03 LAG	Amino Acid Sequence of Mouse Sperm Lactate Dehydrogenase	174
Z01 ES 60064-03 LAG	Primary Structure Characterization of Mouse Muscular Lactate Dehydrogenase	176
Z01 ES 60087-02 LAG	Biochemical Studies of Mouse Enzymes, II. PGK-B Variants	178
Z01 ES 60091-02 LAG	Biochemical Studies of Mouse Enzymes and Genetic Variants, I. Phosophoglucose Isomerase (PGI)	180
Z01 ES 60092-02 LAG	Biochemical Mutations in Drosophila by Chronic Irradiation	182
Z01 ES 60094-02 LAG	BrdU-Dye Studies of Meoitic Recombination	184
Z01 ES 60095-02 LAG	Sister Chromatid Exchange Analyses in Rodent Fetal Tissues	186

Project Number	Title	Page
Z01 ES 60096-02 LAG	Comparison of Rates of Induction of Point Mutations and Chromosome Aberrations in Cultured Cells	188
Z01 ES 61001-01 LAG	Sister Chromatid Exchange Studies in Dividing Cells of the Rodent Colon	190
Z01 ES 61002-01 LAG	Induction of Sister Chromatid Exchanges (SCE) by Ethyl Carbamate in Various Mouse Tissues	192
Z01 ES 61003-01 LAG	Biochemical Analyses of Drosophila Null Mutants	194
Z01 ES 61004-01 LAG	Surveys of Genetic Variation Utilizing Two-Dimensional Electrophoresis	196

LABORATORY OF BEHAVIORAL AND NEUROLOGICAL TOXICOLOGY

Project Number	Title	Page
Z01 ES 70800-03 LBNT	Neurochemical Correlates of Behavioral Toxicology	208
Z01 ES 70805-03 LBNT	Neurotoxicological Effects of Polybrominated Biphenyls in Rats and Mice	210
Z01 ES 90000-02 LBNT	Toxicity of Selenium, Alone and in Combination with Silver or Arsenic, in Rats	212
Z01 ES 90001-02 LBNT	Effects of Acrylamide on Food and Water Consumption and Spontaneous Activity of Rats	214
Z01 ES 90002-02 LBNT	Effects of Diet and Food Restriction on Acrylamide Toxicity in Rats	216
Z01 ES 90003-02 LBNT	Effects of Developmental Exposure to Polychlorinated Biphenyls on Neurobehavioral Functioning of Mice	219
Z01 ES 90004-02 LBNT	Neurobehavioral Assessment of Rats Given Carbon Disulfide	221
Z01 ES 90005-02 LBNT	Effects of Equal Doses of Firemaster and 2,4,5,2', 4',5'-Hexabromobiphenyl in Rats	223
Z01 ES 90006-02 LBNT	Neurobehavioral Toxicity of Developmental Exposure to 2,4-Diphenoxyacetic Acid (2,4-D) in Japanese Quail	225

Project Number	Title	Page
Z01 ES 90007-02 LBNT	Neurobehavioral Toxicity of Neonatal Exposure of Rats to Diethylstilbestrol	227
Z01 ES 90008-02 LBNT	Acute and Chronic Neurobehavioral Toxicity of Acrylamide in Rats	229
Z01 ES 90009-02 LBNT	Neurobehavioral Toxicity of Developmental Exposure of Japanese Quail to Microwave Irradiation	232
Z01 ES 90010-02 LBNT	Neurobehavioral Effects of Chronic Polybrominated Biphenyls in Rats	234
Z01 ES 90011-01 LBNT	Behavioral and Morphological Effects of Triethyl Tin in Rats	236
Z01 ES 90012-01 LBNT	Effects of Developmental Exposure to Monosodium Glutamate on the Neurobehavioral Development of Rats	238
Z01 ES 90013-01 LBNT	Neurobehavioral Effects of Repeated Exposure to Kepone in Rats	240
Z01 ES 90014-01 LBNT	Effects of In Utero Exposure to PBB's on Neurobehavioral Development of Mice	242
Z01 ES 90015-01 LBNT	The Effects of Repeated Administration of Acrylamide on Peripheral Sensation Using an Operant Titration Schedule	244
Z01 ES 90016-01 LBNT	The Effects of Repeated Administration of Acrylamide on Motor Responses Under Control of Operant Schedules	246
Z01 ES 90017-01 LBNT	Effects of Organic and Inorganic Derivatives of Heavy Metals on Binding of Spiroperidol to Striatal Membranes	248
Z01 ES 90018-01 LBNT	Effects of Polyacrylamide on Striatal Nonamine Levels and Binding Site Density	250
	LABORATORY OF BIOCHEMICAL GENETICS	
Z01 ES 65002-02 LBG	Mutation Studies Using Hemoglobin and Monospecific Antibodies Against Hemoglobins	259
Z01 ES 65007-02 LBG	Study of Mutation by Using Sperm Specific Enzyme LDH-X	262

Project Number	Title	Page
Z01 ES 65008-01 LBG	Mechanisms of Sperm Morphology Mutations in PL Mice	265
Z01 ES 65009-09 LBG	Detection of Translocation Carriers by DNA Measurement In Sperm	267
Z01 ES 65010-01 LBG	Translocation and Sperm Abnormality Induction by N-Isopropyl- -(2-methylhydrazino)-p-toluamide hydrochloride) in Mouse Spermatogonia	269
Z01 ES 60019-06 LBG	The Use of Male Germinal Tissue in the Detection of Mutational Events	271
Z01 ES 60021-07 LBG	Gene Mutation Indicators in Mice	273

LABORATORY OF ENVIRONMENTAL BIOPHYSICS

Project Number	Title	Page
Z01 ES 50005-05 LEB	Effects of Noise and Ototoxic Agents on Energy Balance and Metabolism in Cochlea	293
Z01 ES 50014-09 LEB	Microwave Exposure Systems and Microwave Dosimetry	297
Z01 ES 50015-05 LEB	Effects of Microwaves on Neural Response	300
Z01 ES 50016-07 LEB	The Effect of Microwave Radiation on the Heart Rate of Embryonic Quail	302
Z01 ES 50017-06 LEB	Effect of 2450 MHz Microwaves on Embryonic Development, Immunology and Fertility	304
Z01 ES 50019-03 LEB	Effect of Ototoxic Insult on Coding of Complexe Signals in the Auditory System	306
Z01 ES 50020-03 LEB	Optical Fiber Motion Detector for Auditory System Measures	309
Z01 ES 50022-03 LEB	Development of Impact Noise Generator for Hearing Loss Studies	313
Z01 ES 50026-02 LEB	Development of Closed System Electroacoustic Transducer for Noise Research	315
Z01 ES 50027-02 LEB	Prenatal Exposure to High Noise Levels on Auditory Thresholds in Guinea Pigs	317
Z01 ES 50028-02 LEB	The Effects of Noise and Drugs on the Electrochemistry of the Cochlea	320

Project Number	Title	Page
Z01 ES 50029-02 LEB	Acridine Spin Labels as Probes for Nucleic Acids and Their Protein Complexes	323
Z01 ES 50030-02 LEB	Spin Labeled Actinomycin-D Analogs as Base Specific Probes for Nucleic Acids	328
Z01 ES 50031-02 LEB	Molecular Mechanisms of Mast Cell Degranulation	331
Z01 ES 50032-02 LEB	Binding of Copper to Human Serum Albumin	336
Z01 ES 50033-02 LEB	Do Nitroaromatic Free Radicals Covalently Bind to Macromolecules?	339
Z01 ES 50034-02 LEB	The Mechanism of the Oxygen-Insensitive Activation of 4-Nitroquinoline-1 Oxide	342
Z01 ES 50035-02 LEB	Interpretation of the ESR Spectra of Spin-labeled Membranes	344
Z01 ES 50036-02 LEB	A Search for Free Radical Metabolites of Polycyclic Hydrocarbons	346
Z01 ES 50037-01 LEB	Effect of 2450 MHz Microwave Radiation on Plasma and Tissue Enzymes of Guinea Pigs	348
Z01 ES 50038-01 LEB	Effects of 2450 MHz Microwave Radiation on Cultured Heart Cells	350
Z01 ES 50039-01 LEB	The Effect of 2450 MHz Microwave Radiation on Mast Cells	352
Z01 ES 50040-01 LEB	Influence of 2450 MHz Microwave Radiation on Cats Subjected to Myocardial Ischemia	355
Z01 ES 50041-01 LEB	Permeability of the Cochlear Partition Assessed by Electrochemical Potential Change During Anoxia	357
Z01 ES 50042-01 LEB	Comparison of Impact Noise and Continuous Noise Effects on Cochlear Function	360
Z01 ES 50043-01 LEB	Effect of Noise on Embryo/Fetal Development in the Guinea Pig	363
Z01 ES 50044-01 LEB	Effect of Different Noise Exposures on Embryo/Fetal Development in the Mouse	365

Project Number	Title	Page
Z01 ES 50045-01 LEB	Relationship of Catecholamine Levels and Fetolethality in Noise Exposed Mice	369
Z01 ES 50046-01 LEB	Mechanisms of Chemically Induced Photosensitivity	371
Z01 ES 50047-01 LEB	Biological Effects of Fluorescent Lighting	374
Z01 ES 50048-01 LEB	Characterization of Lung Lamellar Bodies Using Spin Labels	376
Z01 ES 50049-01 LEB	Spin-Trapping and Direct ESR Studies of Anticancer Quinone Metabolites	379
Z01 ES 50050-01 LEB	ESR Evidence for a Free Radical in the Cis-Trans Isomerization of Furylfuramide	381
Z01 ES 50051-01 LEB	Free Radical Metabolism of Polycyano Compounds	385
Z01 ES 50052-01 LEB	Binding of Chemically Activated Semiquinone Free Radicals from Anticancer Agents to DNA	387
Z01 ES 50053-01 LEB	Binding of Antitumor Drugs to Membranes	392
Z01 ES 50054-01 LEB	The Free Radical Formed Microsomal Incubations Containing CCl_4 and NADPH	396

LABORATORY OF ENVIRONMENTAL TOXICOLOGY

Z01 ES 70001-03 LET	The Teratogenicity of Antiepileptic Drugs and Chemically Related Compounds	428
Z01 ES 70010-03 LET	Study of Developmental Disorders During Organogenesis in a Model Embryo Culture System	431
Z01 ES 70045-03 LET	Molecular Mechanisms of Androgen Mediated Gene Expression in Male Sex Accessory Glands	434
Z01 ES 70047-03 LET	Regulation of Gene Expression During Differentiation	438
Z01 ES 70060-06 LET	Effect of Prenatal Exposure to Foreign Chemicals on Genital Tract Function	440
Z01 ES 70065-03 LET	The Role of Chemical-Receptor Interactions in Reproduction and Transplacental Toxicity	445

Project Number	Title	Page
Z01 ES 70068-02 LET	Correlative Morphological Studies of Abnormal Cell Differentiation	449
Z01 ES 70070-03 LET	Transplacental Pathology of Environmental Chemicals	452
Z01 ES 70080-06 LET	The Study of Toxic Effects of Environmental Chemicals on Spermatogenesis	455
Z01 ES 70085-02 LET	Development of *In Vitro* Models for Assessing Reproductive Toxicity	461
Z01 ES 70120-08 LET	Hepatotoxicity of Hormonally-Active Chemicals	464
Z01 ES 70132-01 LET	Gastrointestinal Function and Toxicology	470
Z01 ES 70135-03 LET	The Effect of Hormones and Xenobiotics on the Regulation of Perinatal Enzyme Development	475
Z01 ES 70200-06 LET	The Morphologic and Biochemical Effects of Toxic Metals	479
Z01 ES 70210-02 LET	Effects of TCDD on the Disposition of Heme and Heme-Containing Proteins in Mammalian Tissues	483
	LABORATORY OF MOLECULAR GENETICS	
Z01 ES 60052-03 LMG	Environmental Mutagenesis Test Development Program	499
Z01 ES 60077-02 LMG	Molecular Mechanisms of Heat-Induced Mutation	502
Z01 ES 60078-02 LMG	T4 DNA Polymerase (Gene 43) Error Rate with DNA Containing 2-Aminopurine	505
Z01 ES 60080-02 LMG	Modifications of Site-Specific Mutation Rates by a Nearby Base-Pair Substitution	507
Z01 ES 60081-02 LMG	The Genetics of the Misrepair Mutagenesis System of Bacteriophage T4	509
Z01 ES 60082-02 LMG	Neoguanosine as a Naturally Occurring Mutagenic Intermediate	511
Z01 ES 60084-02 LMG	Fidelity of Templated Polymerizing Enzymes During Polymerization	513

Project Number	Title	Page
Z01 ES 60101-01 LMG	Selection of a Computerized Data Base Management System	516
Z01 ES 60102-01 LMG	Testing of Chemicals of Interest in Salmonella	518
Z01 ES 60103-01 LMG	Development of New and Improved Salmonella Test Methods	520
Z01 ES 60104-01 LMG	A Volatile Mutagen from Sodium Azide	522
Z01 ES 60105-01 LMG	Genetic Control of Mutation	524
Z01 ES 60106-01 LMG	Cytogenetic Analysis of Mutagen-Sensitive Mutants	526
Z01 ES 60107-01 LMG	Molecular Cloning and Sequence Analysis of Various Regions of the T4 Genome	528
Z01 ES 60108-01 LMG	Mechanisms of DNA Replication in Prokaryotes	530
Z01 ES 60109-01 LMG	Mechanism of DNA Replication in Mitochondria from Drosophila Eggs and Embryos	533

LABORATORY OF PHARMACOKINETICS

Project Number	Title	Page
Z01 ES 80009-08 LPK	Pharmacokinetic and Pharmacodynamic Functions of the Lung	551
Z01 ES 35003-01 LPK	Pharmacokinetic Parameters in Carcinogenesis: Their Alteration by Anti-carcinogenic Agents	555
Z01 ES 35004-01 LPK	The Metabolism and Disposition of Halogenated Alkyl Phosphates	558
Z01 ES 80014-06 LPK	Metabolism of Polychlorinated Biphenyls	561
Z01 ES 80016-06 LPK	Pharmacokinetics of Chlorinated Xenobiotics	564

LABORATORY OF PHARMACOLOGY

Project Number	Title	Page
Z01 ES 80001-07 LP	Microsomal Mixed-Function Oxidase Systems: Specificity and Function	579
Z01 ES 80002-09 LP	Enzymes Metabolizing Chemicals: Chemical and Physiological Effectors of These Systems	582
Z01 ES 80003-06 LP	Xenobiotic-Metabolizing Enzyme Activity in Skin and Its Response to Environmental Agents	585

Project Number	Title	Page
Z01 ES 80005-06 LP	*In Vitro* Metabolism of Xenobiotics by Selected Marine Species	587
Z01 ES 80006-08 LP	Environmental Contaminants: Uptake, Distribution, Metabolism, Excretion, and Storage Sites in Marine Species	595
Z01 ES 80007-08 LP	Hepatic and Extrahepatic Conjugation and Oxidation Metabolic Pathways for Xenobiotics in Mammals	599
Z01 ES 80031-03 LP	Role of Altered Membrane Function in Xenobiotic Toxicity	607
Z01 ES 80032-03 LP	Excretion of Xenobiotics by Selected Marine and Terrestrial Species	610
Z01 ES 80036-01 LP	Microsomal Mixed-Function Oxidase System: Protein Chemistry	615
	LABORATORY OF PULMONARY FUNCTION AND TOXICOLOGY	
Z01 ES 80033-03 LPFT	Synthesis of Polypeptide Hormones and Prohormones by Normal and Neoplastic Lung Epithelium	623
Z01 ES 25005-02 LPFT	Synthesis and Release of Polypeptide Hormones by Mast Cells	627
Z01 ES 80008-05 LPFT	Biosynthesis, Release, Transport and Metabolism of Prostaglandins (PGs) in Lung	631
Z01 ES 80035-03 LPFT	Co-oxidation of Xenobiotics by the Prostaglandin and Thromboxane Synthetase	635
Z01 ES 25005-01 LPFT	Prostaglandins in Tumor Promotion	638
Z01 ES 25006-02 LPFT	Studies on Thromboxane Synthetase	640
Z01 ES 25006-01 LPFT	The Lung as an Endocrine Organ Controlling Intravascular Thrombosis	643
Z01 ES 80023-06 LPFT	The Composition and Origins of the Acellular Lining Layer of the Lung	647
Z01 ES 80029-03 LPFT	Investigations of Human Pulmonary Disease	650
Z01 ES 25007-01 LPFT	Particle Translocation in Various Cell Types and Anatomic Regions of the Lung	653

Project Number	Title	Page
Z01 ES 25008-01 LPFT	Elemental Analysis of Asbestiform Minerals	655
Z01 ES 25009-01 LPFT	Structure and Function of the Clara Cells Lower Airways	658
Z01 ES 25010-01 LPFT	Effects of Perinatal Exposure to Gaseous Pollutants on Lung Development	661
Z01 ES 25004-02 LPFT	Establishing Pulmonary Epithelial Cells in Culture	664
Z01 ES 25000-02 LPFT	Cultured Epidermal Cells as a Model for Skin Carcinogenesis	667
Z01 ES 25001-02 LPFT	Cellular and Molecular Mechanisms of Neoplastic Progression	670
Z01 ES 25002-02 LPFT	*In Vitro* Carcinogenesis and Promotion Studies	675
Z01 ES 25003-2 LPFT	Tumor Induction Studies with the Tracheal Transplant Model	677

COMPARATIVE MEDICINE BRANCH

Project Number	Title	Page
Z01 ES 30008-08 CMB	The Effects of Environmental Chemicals on Host Resistance to Infectious Agents	696
Z01 ES 30078-02 CMB	Health Surveillance of Laboratory Animals at NIEHS	698
Z01 ES 30080-02 CMB	Simplified Gastric Intubation in the Rabbit	701
Z01 ES 30081-02 CMB	The Effect of Dietary Protein on Lifespan, Frequency of Nephritis and Degree of Hypertension in the Golden Syrian Hamster	702
Z01 ES 30082-02 CMB	Comparison of Hamster Growth Associated with Hoarding and the Inability to Hoard Food	704

ENVIRONMENTAL BIOLOGY BRANCH

Project Number	Title	Page
Z01 ES 80020-07 EBB	Changes in Mammalian Pulmonary Function Produced by Inhaled Environmental Agents	714
Z01 ES 30038-02 EBB	An Evaluation of Contribution of Chlorinated Dibenzodioxins to Hexachlorobenzene Toxicity	717
Z01 ES 30042-04 EBB	Effects of Environmental Contaminants on Cardiac Function	719

Project Number	Title	Page
Z01 ES 30045-03 EBB	Development of an Automatic Small Animal Inhalation Facility	724
Z01 ES 30073-02 EBB	Efforts to Enhance the Elimination of 2,3,7,8-Tetrachloro-dibenzo-p-dioxin from the Body of Rats	727
Z01 ES 30075-02 EBB	The Comparative Toxicity of Technical vs Pure Pentachlorophenol in Dairy Cattle	729
Z01 ES 30077-02 EBB	Dose Response to the Carcinogens, 1,2-Dimethylhydrazine (DMH) and Methylazoxymethanol (MAM) in Rats	731
Z01 ES 30084-02 EBB	Dose-response of Drug-metabolizing Enzymes to Halogenated Biphenyls and Napthalenes	733
Z01 ES 30085-02 EBB	Early Detection of Lung Injury	735
Z01 ES 30088-01 EBB	Toxicity Bioassay of Chlorodecone (Kepone)	739
Z01 ES 30089-01 EBB	Efforts to Enhance the Elimination of Polybrominated Biphenyls (Firemaster FF-1) from the Body of Rats	741
Z01 ES 30090-01 EBB	Toxicologic Evaluation of Pentachloranisole	743
Z01 ES 30091-01 EBB	A Comparison of Low Level Exposure of Polybrominated Biphenyls (Firemaster FF-1) in the Diet vs Gavage in Male CDF Rats	745
Z01 ES 30092-01 EBB	The Effect of Biphenyls on Bone Marrow Cellularity and Progenitor Cells	748
Z01 ES 30093-01 EBB	Macrophage Function and Environmental Toxicology	750
Z01 ES 30094-01 EBB	Effects of Polychlorinated Biphenyl Isomers on Extrahepatic Metabolism of Benzpyrene	752
Z01 ES 30095-01 EBB	Effects of Cutaneous Administration of Technical Pentachlorophenol on Hepatic Enzymes	754
Z01 ES 30096-01 EBB	Use of Whole-Body Sagittal Histologic Sections in Teratologic Studies	756
Z01 ES 30097-01 EBB	Fetal and Maternal Toxicity of 3,3',4,4',5,5'-Hexachlorobiphenyl in the Mouse	759

Project Number	Title	Page
Z01 ES 30098-01 EBB	Tumorigenic Potential of NO_2 Inhalation in Small Animals Exposed to Heterocyclic Amines	761

ENVIRONMENTAL CHEMISTRY BRANCH

Project Number	Title	Page
Z01 ES 30003-07 ECB	Development of Analytical Methodology	781
Z01 ES 30012-06 ECB	Effects of Pure Isomers of Hexachlorobiphenyls on Lipid Metabolism and Cell Structure	783
Z01 ES 30015-05 ECB	Studies in Chemical Ionization Mass Spectrometry	785
Z01 ES 30018-06 ECB	Ester Hydrolases at the Environmental Interface	787
Z01 ES 30020-08 ECB	Transport and Metabolism of Phthalate Esters	789
Z01 ES 30034-04 ECB	Chemistry of Aromatic Compounds and Their Environmental Transformation Products	791
Z01 ES 30035-04 ECB	Chemical Techniques and Spectroscopic Methods	794
Z01 ES 30041-04 ECB	Immunochemistry of Hydrophobic Haptens	796
Z01 ES 30050-03 ECB	Chemical and Enzymatic Conjugation of Glutathione with Arene Oxides and Epoxides	799
Z01 ES 30051-03 ECB	Characteriztion of Specific Binding Modes of Organics and Inorganics: The Toxic Polyhalogenated Aromatic Hydrocarbons	802
Z01 ES 30052-03 ECB	^{13}C-Nuclear Magnetic Resonance Study of Chlorinated Polycyclodiene Pesticides	805
Z01 ES 30058-02 ECB	Synthesis of Thromboxanes and Thromboxane Analogs	807
Z01 ES 30059-02 ECB	Development of New Synthetic Procedures and Reactions	809
Z01 ES 30060-02 ECB	Structure of Fungal Metabolites	811
Z01 ES 30061-02 ECB	Chemical Characterization of Potentially Complex Toxicological Mixtures: Yusho Oil and Used Transformer Fluids	814

Project Number	Title	Page
Z01 ES 30062-02 ECB	Immunochemistry of Dioxin Action on the Lymphocyte and Other Halogenated Aromatic Hydrocarbons	816
Z01 ES 30063-02 ECB	Molecular Orbital Calculations on Molecules of Environmental Interest	818
Z01 ES 30064-02 ECB	Field Desorption Mass Spectrometry Studies	820
Z01 ES 30065-02 ECB	Studies of Mass Spectral Reactions in Field-Free Regions	822
Z01 ES 30066-02 ECB	Development of Synthetic Methods for Polyhalogenated Aromatics	825
Z01 ES 30067-02 ECB	Characterization of the Binding of Heavy Metals in Biological Systems	827
Z01 ES 30072-02 ECB	Effects of Polybrominated Biphenyls: Immune Response in Rodents	829
Z01 ES 30087-01 ECB	The Chronic Toxicity of Technical and Analytical Pentachlorophenol in Cattle: Chemical Analyses of Tissues	831
Z01 ES 30074-01 ECB	Development of Program in Bioinorganic. Chemistry: Phase I-Literature Survey	833

INDEX TO CONTRACT NARRATIVES

Contractor	Contract Number	Title	Page
Arizona Board of Regents	78-2130	Pharmacokinetics of Xenobiotics	573
Battelle Columbus Laboratories	78-2151	Comparative Carcinogenicity and Toxicity Studies of Selected Environmental Chemicals in Laboratory Animals Exposed During Pre- and Postnatal Life	767
Battelle Pacific Northwest Laboratories	78-2150	Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants	102
Becton-Dickinson and Company	74-2164	Effects of Chronic Exposure to Airborne Environmental Agents	765
Brookhaven National Laboratory	Interagency Agreement	Chemical and Environmental Mutagen Studies Utilizing the Tradescantia Test System	198
Case Western Reserve University	79-2136	Microbial Mutagenesis Testing	538
EG&G Mason Institute	79-2137	Microbial Mutagenesis Testing	538
Endocrine Labs of Madison	76-2111	Effects of Environmental Agents on Male and Female Reproductive Tract Function	486
Fisheries Research Institute	77-2101	Carcinogenic Effects of Petroleum Hydrocarbons on Selected Marine and Estuarine Organisms	492
Fred Hutchinson Cancer Research Center	77-22	Statistical Development of Multistage Carcinogenesis Models	158

Contractor	Contract Number	Title	Page
Georgia Institute of Technology	75-2135	Development of a Polygenic Assay for Point Mutations in Mice	278
Hazelton Laboratories America, Inc.	75-2158	Long Term Study on the Biological Effects of Ingested Asbestos in Rats	764
IIT Research Institute	78-2115	Radiosynthesis of ^{14}C-2,3,7,8-Tetrachlorodibenzofuran (TCDF)	838
Illinois Institute of Technology Research Institute	75-2157	Long-Term Study on the Effect of Ingested Asbestos in Hamsters	763
The Jackson Laboratory	74-2156	Development of a System for Detecting Lethal Mutations in Mice	535
The Jackson Laboratory	75-2159	Development of a New Mouse Strain to Maximize the Sensitivity of a Point Mutation Assay	275
Lawrence Livermore Laboratory	78-0038	Survey of Cooked Foods for Mutagenic Activities	536
Lawrence Livermore Laboratory	Interagency Agreement	Cytophysical Studies in Mutagenesis	282
Midwest Research Institute	78-2153	An Appraisal of Environmental Exposure to Heavy Metals	105
National Marine Fisheries Service	Interagency Agreement	The Physiological Effects of Arsenic, Cadmium, and Copper on Marine Shellfish	490
New York University		Photodegradation of Absorbed Polycyclic Arenes	409

Contractor	Contract Number	Title	Page
Oak Ridge National Laboratory	Interagency Agreement	Environmental Mutagen Information Center	276
Oak Ridge National Laboratory	Interagency Agreement	Environmental Teratology Information Center	771
Radian Corporation	78-2144	Chemical Repository for Mutagenicity Screening	537
Research Triangle Institute		Investigation to Determine the Peripheral and Central Receptor Mediating Effects of Microwave Radiation on Brain Activity	408
Science Applications, Inc.	78-2105	Analysis of Total Organic Chlorine and Bromine Residues in Human Body Fluids and Tissues	835
SRI International	79-0001	Microbial Mutagenesis Testing	538
Stanford Research Institute	78-2154	Test Battery for the Neurobehavioral Assessment of Potential Neurotoxins	252
University of Arizona	77-2111	Extrapolation of PCB Disposition	567
University of British Columbia	77C-0604CC	Testing Compounds for Mutagenic Activity in a Meiotic Non-disjunction Test in Neurospora Crassa	200
University of California at Los Angeles	Interagency Agreement	Effects of 60 Hz Fields on the Mammalian Central Nervous System	398
University of Miamai		Protracted Noise Exposure and Cardiovascular Function	403

Contractor	Contract Number	Title	Page
University of Oregon Health Sciences Center	77-2126	Pharmacokinetics of Xenobiotics	571
University of Utah	76-2146	Effects of Microwave Radiation on the Nervous System	401
University of Washington	74-2151	Detection of Point Mutations in Somatic Cells	279
University of Washington	76-2113	Effects of Microwave Radiation on the Nervous System	405
US Energy Research and Development Administration	Interagency Agreement	Susceptibility of Preneoplastic Epithelial Lesions to Toxic and Carcinogenic Insults	679
Virginia Commonwealth University	76-2117	A Study of the Pharmacokinetics and Toxicity of Kepone in Humans and Laboratory Animals	569
Warf Institute, Inc.	77-2141	Analysis of PCB's and DDE in Human Body Fluids and Tissue	836

OFFICE OF THE DIRECTOR

OFFICE OF THE DIRECTOR
Summary Statement

In the past year, the pace of significant change, development, and challenge has accelerated for the National Institute of Environmental Health Sciences. We near completion of our new facility, with its substantially expanded research capacity. At the same time, our staff is nearing the level at which all the various interrelated disciplines are present in sufficient force to begin confronting some of the important environmental health questions on a broad front.

Organizationally, the Institute has filled several critical staff positions with scientists who have the highly specialized training, experience and the breadth of perspective to meet our emerging needs. Both the flexibility and capability of my own office have been increased by the arrival of our Deputy Director, Dr. Robert A. Goyer. Dr. Goyer will greatly strengthen NIEHS management. He is a former Departmental Chairman and his recognized research expertise, especially in pathology and toxicology of heavy metals, will prove invaluable.

In addition, Dr. Charles E. Carter has recently joined the Institute as Scientific Director, responsible for the Intramural Research Program. Dr. Carter, a widely recognized research scientist, represents a continuance of the kind of leadership that will help guide us as we enter our second decade as an Institute. He will continue the kind of critical yet innovative administration needed to make our research both excellent in itself and well-matched to our mission in public health.

Other developments have enhanced our capabilities as an Institute. Reorganizations have created separate Chemistry and Environmental Biology Branches within the Research Resources Program. In the Intramural Program a new Laboratory of Organ Function and Toxicology has been created from a section within the Laboratory of Environmental Toxicology. The subdivisions reflect the specialization and development of two important areas within environmental health sciences: (1) chemistry, both as a support activity and as an area for basic research and, (2) the target organ approach to toxicology. The subdivision also enables laboratory chiefs of the pre-existing labs and of the new labs to limit their administrative responsibilities so that their attention can be more closely directed toward the research itself.

In the past year, an active area in terms of organizational change and development has been environmental mutagenesis, the study of the effects of environmental agents on the chromosome and gene structures which determine at conception the eventual overall health of the future adult person. This area of investigation has proved to be inseparably related to understanding the toxicity of environmental agents; and in terms of its importance, relatively unexplored. For this reason, we have sharpened our focus in mutagenesis by dividing the Laboratory of Environmental Mutagenesis into two laboratories, namely: the Laboratory of Molecular Genetics and the Laboratory of Animal Genetics. These two will work within their special areas of emphasis and in concert with each other, and with the Laboratory of Biochemical Genetics.

This resume of our ongoing research will give a laboratory-by-laboratory account of achievements and work in progress, within the limits of space. A few examples at the start will illustrate the range of efforts that are building into a more comprehensive basic science program in the environmental health sciences.

The possible effects of low-level PCB exposure resulting from ingestion of mother's milk are being monitored to determine if PCB's pose any hazard to offsprings who nurse. The children are being evaluated over the first six months of life with close attention to their growth and development, and they will be followed until school age in order to identify and assess any possible learning or behavior problems.

Increased attention to the chemical insults experienced over a lifetime by the human organism has encouraged a more comprehensive approach to the area of toxicology in public health. The Secretary of DHEW has established the National Toxicology Program (NTP) which will coordinate toxicological research, test development, and test validation throughout DHEW and components devoting specific blocks of resources to NTP. NIEHS joins with FDA, NCI, and CDC/NIOSH in this endeavor.

The Director, NIEHS, serves as the Director of the National Toxicology Program. NIEHS is currently involved in the development of a group of tests that will screen substances through successive test systems for different kinds of toxicities--mutagenicity, carcinogenicity, teratogenicity, effects on the immune and reproductive systems, and so forth. This calls for the most flexible, sensitive, scientifically sound, and cost-effective approach to testing that can possibly be devised.

Also, the Director, NIEHS, serves as the Chairman of the DHEW Committee to Coordinate Environmental and Related Programs (CCERP). This multi-agency group provides a medium to assure the exchange of information on environmental health, toxicology, and related programs; to coordinate these programs; to enhance sharing of resources and to provide advice to the Department.

Both as a part of the Institute's mutagenesis program and as a part of the Institute's role in the National Toxicology Program, we have devoted staff and resources to the Environmental Mutagenesis Test Development Program. The importance of testing for mutagenicity is suggested by the close relationship between those agents that cause mutations and those that cause cancer. Further, we know that cancer is not the only negative health impact of mutagenicity. Other probably effects include chronic and metabolic dieseases, birth defects, defects in the immune system, nervous and behavioral disorders and so on.

In the Institute's Environmental Chemistry Branch, radioimmunoassay techniques have been developed to identify specific environmental agents and determine their levels down to the parts per trillion range. This system utilizes equipment and know how already available at almost all hospitals.

In the Environmental Biology Branch, research on the wood preservative and agricultural product pentachlorophenol revealed that specific contaminants (dioxins) in the commercial product--and not the chemical itself--produced the over toxicity that, among other manifestations, caused cattle to get sick or gain wieght much more slowly than normal.

Other Institute research followed up human exposures to lead and mercury, increasing the knowledge of the kinds of toxicity these agents produce. The Mount Sinai School of Medicine, with core support from the Institute, investigated occupational exposure to lead among indoor firing range marksmen and instructors. Their report on elevated lead levels among this group stimulated a private ammunition manufacturer to develop a nylon-jacketed bullet which when used greatly decreases the lead exposure to indoor firing range users.

These are some examples of the Institute's many scientific investigations probing the relationship of environmental effects to human health. Every investigation seeks to answer a specific question or group of questions, and the data gathered and the conclusions drawn serve to broaden the area of possible inquiry.

Now modern science has come to the place where we are learning that we must respect life's delicate requirements for sustaining itself. We must study these requirements with our greatest scientific and intellectual intensity. From this perspective, the discoveries that we are making about life's relationship to the rest of our world are not bad news, but promising news that will enable us to live harmoniously with our environment.

EXTRAMURAL PROGRAM

OFFICE OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL PROGRAM
Summary Statement

During FY-79, 511 research applications were reviewed, an increase of 75 applications over FY-78. From these applications, 136 awards were made. Further breakdown shows that 103 regular research grants, 1 Environmental Health Sciences (EHS) Center, 1 Marine and Freshwater Biomedical (MFB) Center, 2 Institutional National Research Service Awards (NRSA), 23 Individual NRSAs, 3 Research Career Development Awards (RCDA) and 3 Academic Investigator Awards were funded. These new and competing awards plus the non-competing awards increase the National Institute of Environmental Health Sciences (NIEHS) active grants to 460.

The Young Environmental Scientist (YES) Program, now in its second year, continues to encourage applications from recent graduates who have expressed interest in carrying out research in the broad field of environmental health sciences. The program now includes 21 grants in 20 institutions in the areas of prediction, detection, and assessment, and mechanism of environmentally-caused diseases and disorders. Feedback from applications indicates that indeed this program has stimulated young investigators to apply for support from the NIEHS in the field of environmental health sciences.

The RCDA Program sponsors 15 research investigators, 13 of whom hold the Ph.D. degree, and 2 the M.D. degree. Departmental affiliation includes: biochemistry, biology, biostatistics, chemical oceanography, chemistry, entomology, environmental medicine, environmental sciences, environmental sciences and engineering, medicine, nutrition and food science, oncology, otolaryngology and communication sciences, and pharmacology. The high level of research activity of the RCDA recipients is expressed in the large number of publications and papers presented in the progress reports.

EHS Centers, which are University-based, comprise an essential national resource and are an integral part of the strategy of NIEHS in serving the national needs in environmental health research. These Centers perform high quality, multidisciplinary research in areas of high relevance to the mission of NIEHS and serve to attract and train outstanding students to meet future research needs of the Nation. During FY-79, the NIEHS supported 9 EHS Centers with funds totaling $8,907 million.

Because of the importance of the EHS Centers in meeting National needs, communication between the Centers, the Institute Director and his staff, and the National Advisory Environmental Health Sciences Council (NAEHSC) is maintained at an effective level through biannual meetings of the Center Directors, frequent visits and telephone contacts by Extramural staff. Members of the NAEHSC Subcommittee for Environmental Health Sciences Centers frequently accompany staff on oversite visits, as well as attending Center Directors' Meetings. The Subcommittee provides liaison between the Institute, the Advisory Council, and the Center Directors by keeping abreast and discussing research problems and accomplishments, new environmental concerns, and program directions.

The 19th meeting of the EHS Center Directors was held at the Oregon State University, Corvallis, Oregon, on October 19-20, 1978 with Dr. Virgil Freed as host. The first day of the meeting was devoted to presentations by staff of the host center. Dr. David Rall, Director, NIEHS, and Dr. Jack Moore, Associate Director Research Resources Program, discussed the proposed National Toxicology Program; its relationship to the NIEHS, the National Institutes of Health's Bureaus, Institutes, and Divisions, and other Federal agencies at the evening session. The second day of the meeting was devoted to 30 minute presentations by Directors and/or Senior staff of ongoing research at the other Centers.

The 20th meeting of the EHS Center Directors was held June 17-19 at the New York University Medical Center, New York, with Dr. Norton Nelson as host. Sunday evening, Congressman Ambro, Chairman of the Committee on Natural Resources and the Environment, addressed the group on research and planning needs in environmental health and the interests and concerns of his committee in this area.

Monday, Dr. Nelson and staff presented brief summaries of their research activities. Tuesday was devoted to presentations by the Directors of the other 8 EHS Centers.

The first meeting of the Directors of the MFB Centers was held April 11 at NIEHS. The morning session included presentations by NIEHS Intramural scientists on research activities of interest to MFB center staff. The afternoon session was devoted to presentation of research activities at, and administrative structure of, the 4 individual MFB Centers. These meetings will be scheduled on an annual basis and will be hosted by the NIEHS and individual centers on a rotating schedule.

One new EHS Center and 1 new MFB Center were funded in FY-79.

The EHS Center is located at the University of California, Berkeley. This group of scientists, under Dr. Ames, has made a major contribution in the area of environmental mutagenesis through the development of a sensitive mutagenic test system.

The new MFB Center is located at the University of Seattle under the direction of Dr. Earl Benditt. The research interests of this Center's staff concern the mechanisms of action of teratogenic, mutagenic, and carcinogenic agents on aquatic systems. This brings to 5 the number of MFB Centers funded by NIEHS.

RESEARCH MANPOWER DEVELOPMENT SECTION

Training Program

The NIEHS Extramural Training Program is operated under the authority of the National Research Service Awards Act which provides for the support of pre- and postdoctoral training by institutional awards and postdoctoral fellowships. On November 9, 1978 this authorization was extended through Fiscal Year 1981 incorporating the following program changes.

- The limitation on individual fellowship awards was revised downward to not less than 15% of dollars appropriated, but not less than 50% of total funds shall be made available for institutional program awards.
- Short-term training awards are authorized. Made for 3 months or less the total cost may not exceed 4% of the amount obligated under the National Research Service Award Act. This training is also exempt from payback requirements.
- The requirement for National Advisory Council review of individual Fellowship awards has been deleted, thereby reducing the review period by about four months.
- Individuals may receive five years of aggregate NRSA support at the predoctoral level and three years aggregate at the postdoctoral level without a waiver request.
- Provisions have been made for periodic cost of living adjustments which will likely result in future stipend increases.
- Certain payback provisions have been modified.
- The current annual report from the National Academy of Sciences on biomedical manpower needs now will be required only every two years.

It is the goal of the NIEHS Training Programs to train individuals for careers generating new knowledge in the biomedical aspects of environmental health sciences. These individuals will, through research, promote better comprehension of current and potential hazards to man of exposure to environmental agents.

The following four areas of training fall within the scope and authority of the NIEHS:

Environmental Toxicology: Training in this area focuses on didactics and research experiences in toxicological principles which determine the effects on organs and tissues of exposures to environmental agents. Pharmacokinetic and pharmacodynamic factors, cellular and molecular mechanisms of action, synergism, species variability in toxic response, and test development, design and interpretation are all facets of this training area. Graduates are qualified to pursue careers in toxicology in academia, industry or government.

Environmental Pathology: Training in this area focuses on factors involved in chemical (as opposed to infectious disease) pathology. Typically, trainees and fellows hold professional or academic degrees which qualify them for advanced training in gross- and histopathological research dealing with the structural and functional alterations of tissues exposed to environmental chemicals. Graduates are qualified to become members of research teams involved in chemical risk evaluation using experimental models where their primary roles will be to lead in the pathological phases of research and to assist in experimental design and interpretation, and/or to initiate independent research on the functional and morphological consequences of environmentally induced diseases.

Environmental Mutagenesis: Training in this area emphasizes the application of the basic principles of genetics and biochemistry to applied studies aimed at assessing the potential genetic hazards to man of exposure to environmental chemicals. Research on the basis for chemically induced mutations is stressed. Graduates enter careers aimed at understanding hazards and developing predictive tests in this field.

Environmental Epidemiology and Biostatistics: Training in epidemiology teaches the utilization of statistical and mathematical tools to assist in the identification of environmental diseases in human populations. The focus is on non-infectious disease epidemiology with an emphasis on the identification of causes of environmental disease. Biostatistics training teaches the application of mathematical and statistical tools to assist environmental health scientists in experimental design and interpretation. These programs provide research epidemiologists and statisticians to analyze data from human or animal populations to determine the potential human hazards of exposure to environmental agents.

The following institutions are supported for training in these areas:

ENVIRONMENTAL TOXICOLOGY

	INSTITUTION	DIRECTOR
1.	Johns Hopkins University School of Hygiene & Public Health Division of Toxicology Department of Environmental Health Sciences	Robert J. Rubin (Pre/Post)
2.	University of California, Davis Department of Environmental Toxicology	James N. Seiber (Pre/Post)
3.	University of Cincinnati Department of Environmental Health	Paul B. Hammond (Pre only)
4.	Duke University Medical Center Physiology & Pharmacology Department	Daniel Menzel (Post only)

5.	University of Wisconsin Bacteriology Department Madison Center for Environmental Toxicology	Ronald Hinsdill (Post only)
6.	Massachusetts Institute of Technology Nutrition and Food Science Department School of Sciences	Gerald Wogan (Pre/Post)
7.	University of Illinois Biology & Entomology Department Institute for Environmental Studies	Robert Metcalf (Pre/Post)
8.	Vanderbilt University School of Medicine Department of Biochemistry	Robert Neal (Pre/Post)
9.	Medical College of Wisconsin Department of Pharmacology	James Fujimoto (Pre/Post)
10.	University of Mississippi Medical Center Department of Pharmacology and Toxicology	A. Wallace Hayes (Pre/Post)
11.	North Carolina State University Department of Entomology	Frank E. Guthrie (Pre/Post)
12.	Children's Hospital Research Foundation Pediatrics Department	Ernest Zimmerman (Pre only)
13.	University of Rochester Pharmacology and Toxicology Department School of Medicine and Dentistry	Victor G. Laties (Pre/Post)
14.	Duke University School of Medicine Pulmonary Medicine Division Medicine Department	William Lynn (Pre/Post)
15.	Cornell University Department of Entomology	Chris Wilkinson (Pre/Post)
16.	New York University Department of Environmental Medicine	Morton Lippman (Pre/Post)
17.	Albany Medical College Center of Experimental Pathology and Toxicology	Rajender Abraham (Pre/Post)
18.	Michigan State University College of Osteopathic Medicine Carcinogenesis Laboratory	J. Justine McCormick (Post only)
19.	New York University Medical Center Department of Environmental Medicine	Roy E. Albert (Pre/Post)

20.	Purdue University School of Pharmacy & Pharmacology Sciences	John E. Christian (Pre only)
21.	Oregon State University Environmental Health Sciences Center	Ian J. Tinsley (Pre/Post)
22.	University of Kansas Medical Center College of Health Sciences	Curtis Klaassen (Pre/Post)
23.	Thomas Jefferson University Jefferson Medical College	C. Paul Bianchi (Post only)
24.	Yale University School of Medicine	Margaret Hitchcock (Post only)
25.	Medical College of Virginia Department of Pharmacology	Albert Munson (Pre/Post)
26.	University of Texas Health Sciences Center Medical School & School of Public Health	Sheldon Murphy (Pre/Post)
27.	University of Arizona Health Sciences Center	J. Wesley Clayton (Pre/Post)
28.	Johns Hopkins University School of Hygiene & Public Health	Zoltan Annau (Pre/Post)
29.	Utah State University Toxicology Program	Joseph Street (Pre/Post)
30.	University of Michigan School of Public Health	Herbert Cornish (Pre/Post)
31.	Roswell Park Memorial Institute New York State Department of Health	Harold Box (Pre/Post)

ENVIRONMENTAL EPIDEMIOLOGY AND BIOSTATISTICS

1.	University of North Carolina Department of Biostatistics	Lawrence Kupper (Pre/Post)
2.	Harvard University Department of Epidemiology	Richard Monson (Pre/Post)
3.	University of North Carolina Department of Epidemiology	Herman Tyroler (Pre/Post)
4.	Florida State University Department of Statistics	Fred Leysieffer (Pre/Post)

5.	University of Cincinnati Department of Environmental Health	Ralph Buncher (Pre/Post)
6.	University of Illinois School of Public Health	Henry Gelfand (Pre/Post)
7.	University of Pennsylvania School of Medicine	Anita Bahn (Pre/Post)
8.	Yale University School of Medicine Department of Epidemiology and Public Health	Jan Stolwijk (Pre/Post)
9.	University of California - Los Angeles School of Public Health	Roger Detels (Pre/Post)

ENVIRONMENTAL MUTAGENESIS

1.	University of California, Berkeley Department of Biochemistry	Stuart Linn (Pre/Post)
2.	Case Western Reserve University School of Medicine Department of Radiology	Helen H. Evans (Post only)

ENVIRONMENTAL PATHOLOGY

1.	University of North Carolina Department of Pathology	Joseph Grisham (Post only)
2.	University of California, Davis Veterinary Pathology Department	Donald Dungworth (Post only)
3.	University of Washington, Seattle Department of Pathology	N. Karle Mottet (Pre/Post)
4.	Washington University, St. Louis Department of Pathology	Michael Lieberman (Pre/Post)
5.	University of Wisconsin Pathology Department	James Allen (Pre/Post)
6.	State University of New York-Stony Brook Department of Pathology	Philip Kane (Pre/Post)
7.	Mount Sinai School of Medicine Department of Community Medicine	William Nicholson (Post only)

In addition approximately 40 postdoctoral fellows are being supported, with almost all of these individual awards being in the field of environmental toxicology.

Because the NIEHS Training Program is the major federal support mechanism for the production of individuals in these areas, budget for the program continues its growth, demonstrating the Administration's and Congress' interest in maintaining a flow of trained researchers into these fields. The following table presents training budgets and manpower figures showing the recent growth in the program.

	FY 76	FY 77	FY 78	(Est) FY 79	(Est) FY 80
Budget ($1000's)	$1,745	$4,194	$4,568	($4,568)	($6,568)
Students Supported Pre/Post	115/79	182/149	179/176	(175/180)	(250/250)

Finally, the Institute wishes to assess the current supply/demand situation for the individuals trained on these programs. In the last fiscal year a contract was let for such an assessment in the field of environmental toxicology. A comprehensive survey of all institutions training and employing environmental toxicologists is in progress to determine the actual demand for such individuals and the effectiveness of the NIEHS training programs in producing them. With this information, the Extramural Training Program will make any appropriate modifications indicated necessary by this survey.

HIGHLIGHTS OF RESEARCH IN EHS CENTERS

Mount Sinai School of Medicine

The research programs of this Center at the Mount Sinai School of Medicine continues along the lines noted in past years.

Human Health Effects of Long-term, Low-level Exposure to Environmental Agents. Research interest has continued in relation to a number of agents. Studies have been completed on pulmonary disease among titanium workers. Although some changes were found, these did not seem to constitute a significant clinical problem among heavily exposed titanium dioxide workers. This promises well for the much less heavily exposed individuals in the general population, who have been exposed to the metal dust particles as the result of the widespread use of titanium dioxide in paper, paints, and other widely used materials.

There has been important extension of lead research in the past year and studies will continue and expand. An investigation of the status of secondary lead smelter workers in three facilities in Indianapolis and Vernon, California were completed. The findings have provided information for current evaluation of regulation and control of occupational and environmental lead exposure. The research broke new methodological ground, particularly in investigating the relation between zinc protoporphyrin and other biochemical parameters and additional studies broadened appreciation of clinical problems in several areas as lead poisoning among demolition workers and firing range instructors.

During the past year, research activity concerning PCBs was continued. In large part, this has been stimulated by three factors: the increasing recognition of widespread prevalence of measurable PCB tissue burdens in a large proportion of the U.S. population, as evidenced by the identification of virtual universal contamination of human breast milk and by measurable levels of PCB in sera in the general population; the paucity of information concerning the potential for adverse human health effects; and the opportunity to undertake appropriate investigations of the long-term human health effects of defined PCB exposures.

There is extensive experience with animal studies of nitrosamines. Interest in these compounds has been stimulated by the fact that they have been found widely present throughout the environment, including food, industrial chemicals and in the general community air. Additional information has become available on the compounds in a number of cutting oils. A previously established investigation into the mortality experience of a group of workers exposed to dimethylnitrosamine was continued and feasibility studies in two additional directions have been initiated. The clinical status of workers in a plant manufacturing cutting oils, in which nitrosamines had been found, has been evaluated. Briefly, a clinical field survey of 40 workers was conducted; nothing unusual that could be related to chemical exposure was detected.

Studies are continuing on organic fibers along several lines. No unusual mortality patterns were found although there was a curious absence of the "health worker effect" in the first 5-10 years from entrance into the cohort. A feasibility study has been launched into the clinical status of individuals employed in garnetting work.

Fire fighters face a "new fire environment" associated with plastics and chemicals, in addition to previous hazards such as carbon monoxide, heat, fumes, etc. The several fire fighters' unions in New York City, and the New York City Fire Department, are cooperating to determine how best to help in evaluating hazards associated with fire fighting. Access to appropriate records of the Fire Department has been obtained that will permit investigation of the mortality experience of fire fighters in New York City.

Multiple Factor Interaction of Environmental Agents. This program area remains of considerable interest because of its broad theoretical and practical connotations. It is likely that most human cancers, for example, are the result of multiple factor interactions, often agents which do not induce cancer by themselves.

Asbestos-cigarette smoking interaction, first described in 1968, continues to be studied. While the lung cancer risk of cigarette smokers is sharply increased by asbestos exposure, this does not occur with either pleural or peritoneal mesothelioma. The risk of death of asbestosis is increased among asbestos workers who smoke cigarettes, compared to asbestos workers who do not. However, this increase is modest compared to that which occurs for lung cancer. Of interest also is the question whether the decrease in lung cancer incidence among cigarette smokers who stop smoking would also be found among cigarette smoking asbestos workers who stop. Initial studies suggest the incidence is reduced in the latter case also.

Major effort continues to be devoted to the evaluation of human health effects associated with asbestos exposure, especially that at levels below those generally encountered in direct asbestos employment. An important objective is to clarify the scientific basis for public health approaches to the control of environmental asbestos hazards. Extensive, ongoing asbestos research programs may perhaps then be best analyzed in such terms. Many of the research projects are continuing studies primarily devoted to prolonged observation of defined population groups.

It is now 15 years since investigation began of asbestos insulation workers in the New York-New Jersey metropolitan area. 1,522 men were members of this Union at some time between January 1, 1943 and December 31, 1962. Approximately 20% of all deaths appear to be caused by lung cancer, approximately 6% by mesothelioma of the pleura or peritoneum, approximately 7% by asbestosis and 20% by cancers of other sites. During the past year, mortality experience for the years 1943-1974 were analyzed.

A second, larger cohort was established January 1, 1967. It was composed of all 17,800 men on the rolls of the International Association of Heat and Frost Insulators and Asbestos Workers in the United States and Canada on that date. The large number of deaths and the extensive experience which they reflect (more than 160,000 man-years of observation) now permits evaluation of the incidence of less common neoplasms. Statistically significant increased incidence of cancer of the oropharynx, larynx and kidney have been found. On the other hand, incidence of cancer of a number of other sites, including blood, bladder and liver does not appear to have increased. Data are still insufficient to allow firm judgment concerning cancer of the brain or pancreas.

It has been of considerable interest to obtain information concerning the specifics of asbestos exposure which might be related to subsequent asbestos disease. Such attempts bear the constraint of the long period of clinical latency between exposure and subsequent disease. For mesothelioma, lung cancer and extensive asbestosis, this is measured in decades. Even for evidence of limited asbestotic change (reticular fibrosis, pleural fibrosis and/or plaques) 5-15 years generally must pass before the first radiographic signs become apparent. Attempts are being made to obtain data that would refine such early observations but these indications of early change are still comparatively insensitive. Epidemiological studies of groups with exposure with defined limits offer much promise and such research is being explored and new feasibility studies are planned.

As a corollary to these investigations, quantitative measurements are being obtained to further define dose-disease response relationships. Dust exposures among insulation workers have been considered and technical problems in the comparison of optical and electron microscopic measurements have been reviewed. Disease among family contacts is being recorded; dust exposures within such homes are also being analyzed. Asbestos disease associated with dry wall construction and the use of taping and jointing compounds has been recognized. Exposures related to such disease are also being investigated. In the problem of asbestos contamination of schools and other buildings, similar measurements are being sought and in recent probes into environmental asbestos contamination resulting from the use of quarried asbestos-content rock, environmental measurements have played an important role.

A wide variety of basic laboratory studies are in progress, concomitant with the clinical, epidemiological, industrial hygiene and environmental evaluation studies. Surfaces of asbestos fibers are being studied and the influence of the various types of fibers (and other minerals) in causing hemolysis in *in vitro* test systems continued under study. Studies in animal models continue, including a new investigation utilizing the trachea of the rat in an *in vitro* system. The cellular response derived from material in the intact animal has been of interest as has the immuno-competence of patients with mesothelioma. Aryl hydrocarbon hydroxylase in the rat is being studied along with levels of AHH among asbestos workers.

In addition to extensive studies concerning mesothelioma among people exposed to asbestos, specific, more detailed, investigations concerning the neoplasm have been initiated. These include electron microscopy of mesothelioma experimental studies, and the prognostic significance of pleural plaques for mesothelioma and their use as potential markers for asbestos associated neoplasms. Unusual (5-year) survival of a patient with proven mesothelioma has led to close investigation of the individual and an extensive study that will correlate clinical and histological findings in a large group of individuals with the disease. Immuno-competence studies in patients with diffuse mesothelioma will be part of a large scale study to be initiated in the near future using experimental animal models for the investigation of a number of therapeutic approaches. The latter will be part of an overall program for the investigation of improved treatment and management of patients with diffuse malignant pleural or peritoneal mesothelioma.

New York University Medical Center

The Center at New York University can be divided into 5 laboratories.

Research in Toxicology. Work in the metabolism and toxicity of sulfur dioxide and sulfite salts has been continued. Emphasis has been placed on the kinetics of S-sulfonate ($R-S-SO_3^-$) formation resulting from the sulfitolysis of disulfide bonds in plasma and other tissues, and on the toxicological implications of this reaction. The pseudo-first order reaction kinetics of S-sulfonate formation in lung and aorta have been determined during continuous i.v. infusion of sulfite, as well as the biological stability of the S-sulfonate compounds formed in these tissues. It is believed that the reaction of sulfite with certain aorta and lung protein disulfides at critical periods during development may alter the normal maturational pattern of these tissues.

The effect of sulfite on the binding of the neurotransmitters acetycholine and serotonin to their respective receptors in rat brain homogenate has been investigated. It was found that 10 mM sulfite interfered with normal binding of both acetylcholine and serotonin to their respective receptors in rat brain. Interference at lower sulfite concentrations, however, was minimal under these conditions.

The initial phase of the new behavioral toxicology program, which included setting up and detailed testing of new equipment has been completed. New procedures are being developed to reveal impairment of visual discrimination and temporal discrimination, two vital human functions which can be measured chiefly in behavioral terms. Work underway with pigeons should yield general principles for later use with primates. Forced-choice procedures employing repeated measurements are being used because of their applicability for the study of subtle consequences of chronic low-level exposures.

Methodology is being developed for the measurement of stable elements in blood using X-ray fluorescence analysis (XRF). Using the lighter 35 mg/cm^2 samples (reduced from the 80 mg/cm^2 samples used for lead measurements only) a calibration curve has been obtained for the simultaneous measurement of the elements S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Pb and in red blood cells. The sensitivity of the system in terms of minimum detectable levels of these elements, using a 30 min. counting time and a 0.05 significance level ranges from 4 μg/gm for S to 0.01 μg/gm for Br and Pb.

The interaction of polycyclic aromatic hydrocarbon (PAH) carcinogens with the aorta are being investigated in chickens and rabbits. The test compound was the potent PAH carcinogen, 7,12-dimethylbenzanthracene (DMBA). The earlier finding that weekly administration of PAH carcinogens markedly accelerated atherosclerotic plaque formation in the aortas of chickens has been confirmed. A series of dose-response experiments was performed on male and female chickens. Animals received weekly injections of 5, 10 and 20 mg of purified DMBA/kg body weight beginning at 5 weeks of age and continuing for 16 weeks. Even at the lowest dose level, nearly 40% of all males displayed plaques compared to only 15% in the control groups. In general, plaques were larger and more frequent in males than in females.

Chemical Carcinogenesis. The objective of the prevention of environmentally-induced cancer continues to be pursued by the identification of potential cancer hazards extant in the environment, improvement of basic mechanisms at the cellular level which lead to the production of malignant cells, quantitation of the kinetics for tumor formation and development of optimal test systems in animal models for the definition and evaluation of environmental carcinogens.

Ethylene dibromide (EDB), a known stomach carcinogen in mice and rats, and ethylene dichloride (EDC), a compound which causes tumors in the liver and lung of mice, were shown to bind covalently to stomach and hepatic microsomal proteins and to salmon sperm DNA after activation by an in vitro microsomal system. SKF-525A, an inhibitor of cytochrome P-450, significantly inhibited the binding of EDB to protein and DNA. Glutathione and 1-methyl-2-mercaptoimidazole also markedly decreased the binding. The binding of EDC to liver proteins of B6C3F$_1$ mice, a species susceptible to liver tumor induction by EDC, and to DNA was significantly higher than the corresponding binding for Osborne-Mendel rats, a species not susceptible to liver tumor induction by this compound.

The in vitro and in vivo binding of EDC to lung cellular macromolecules is currently under investigation since lung is also a target organ for EDC-induced carcinogenesis. These studies indicate that metabolic activation of EDC is required for the covalent binding to lung microsomal proteins and exogenous DNA and that there is a correlation between the binding of EDC to proteins from lung and EDC-induced lung carcinogenesis.

Studies are underway to isolate and characterize urinary metabolites of EDB from male Osborne-Mendel rats which were given ^{14}C-EDB intragastrically. Thus far two metabolites have been tentatively identified. These are S-(β-hydroxy ethyl)-cysteine and N-acetyl-S-(β-hydroxyethyl)-cysteine. A number of other metabolites are also present and their isolation and characterization is in progress.

The structure of new compounds of interest in carcinogenesis studies has been elucidated. Several chloroalkene epoxides, the potential activated carcinogenic intermediates of chloroalkenes, have been synthesized and characterized. The stability of these reactive compounds was studied with respect to hydrolysis at physiological conditions and thermolysis. A new technique was developed for trapping and characterizing those epoxides too unstable to be detected directly. It employs a nucleophilic agent which reacts with the epoxide forming a stable adduct and structure determination using isobutane chemical ionization mass spectrometry.

Exploratory studies are being carried out to detect highly unstable electrophilic epoxides formed in the atmosphere of New York City by trapping them with a suitable nucleophile. The adducts formed from the reaction of authentic epoxides and sulfhydryl nucleophiles have been isolated in order to determine the feasibility of the procedure. The structures of the adducts were elucidated using nuclear magnetic resonance and chemical ionization mass spectrometry.

Development of procedures for detection of bladder carcinogens and of early changes in the bladder urothelium characteristic for carcinogenesis has continued. Such methods would aid in the diagnosis and prevention of bladder cancer. Cooperative projects are underway with other laboratories studying characteristic features of the carcinogenic process. One study was completed in the past year on the effects of 13-*cis*-retinoic acid, a known inhibitor of bladder cancer, on early changes in the scanning electron microscopic (SEM) features of rat bladders fed the carcinogen, N-[4-(5-Nitro-2-furyl)-2-thiazoyl] formamide (FANFT). 13-*cis*-retinoic acid markedly inhibited the developing hyperplasia induced by FANFT from 1 to 6 months after feeding. A considerable reduction in pleomorphic microvilli was also observed in 6 months. This feature is almost characteristic for a neoplastic cell and our data indicate that SEM may detect inhibition of neoplastic changes long before tumors appear (6 vs. 17 months). An experiment with soy beans, which is in progress, will correlate early SEM changes with any inhibition of FANFT-induced bladder tumors. A low molecular weight protease inhibitor has been observed in snails. This material is being purified for testing as an inhibitor of bladder cancer.

Percutaneous application of chemicals on mouse skin provides a method of measuring initiators of carcinogenesis. Sencar tumor sensitive mice were used in two-stage carcinogenesis tests employing a variety of carcinogens as initiators and phorbol myristate acetate (PMA) as promoter. The tumor yield and time of appearance was compared to Swiss-Millerton mice (ICR). In these tests the initiator was applied at two concentrations and followed three days later by application of PMA for the duration of the experiment. Sencar mice were more responsive to carcinogens requiring metabolic activation than ICR mice. Thus, they responded with multiple tumors in two-stage carcinogenesis to the liver carcinogen acetylaminofluorene, to the bladder carcinogen butyl butyl-hydroxy nitrosamine (BBN) and the flame-retardant TRIS, while ICR mice failed to respond to these agents. They also showed a higher tumor response with dimethyl benzanthracene with significantly lower PMA concentration. On the other hand, Sencar and ICR mice responded identically to the direct alkylating carcinogen beta-propiolactone.

Metals which have been identified as carcinogens in humans through occupational exposure, or in animals through laboratory experimentation are being studied. A number of metals have been reported to be mutagenic in bacteria or phage. These include salts of arsenic, chromium, copper, iron, manganese, molybdenum, plantinum and selenium. However, the mutagenicity of metals is not easily assayed in standard microbial test systems, such as the Ames test. In order to examine the mutagenicity of carcinogenic metals, and to develop a better test system for other highly toxic agents, work at the Institute has stressed the use of metals at non-lethal concentrations, a situation relevant to the actual environmental situation. Mutagenesis testing is accomplished by performing a fluctuation test, whereby multiple samples of small numbers of bacteria undergo many rounds of cell division in the presence or absence of non-lethal concentrations of metals. These samples are then examined for mutants, using three different genetic markers (two forward mutations and one reversion). DNA repair mutants, lacking various repair capacities, can also be used in these tests, and are now under investigation.

Studies on the effects of arsenite on UV-irradiated *E. coli* lead to the conclusion that arsenite is an inhibitor of error-prone DNA repair. This conclusion results from using a variety of DNA repair mutants in these studies. The use of these mutants is a powerful tool for finding agents which enhance or suppress mutagenesis. In these studies, arsenite showed a weak anti-mutagenic effect.

Chromate, another carcinogenic metal, showed a different effect on DNA repair mutants. It inhibited survival under UV-irradiation only in mutants capable of excision repair, suggesting that chromate inhibits excision repair. Using a fluctuation test, chromate was shown to be mutagenic. A comparison between carcinogenic and non-carcinogenic metals is underway.

A new project on the mechanism of DNA repair and its role in carcinogenesis has been initiated. Faulty replication of DNA is believed to play a role in carcinogenesis. Also, studies have indicated that DNA damage can be easily measured in mammalian cells and thereby serve as a simple bioassay for carcinogens. DNA damage and repair in mouse epidermal cells is now being investigated *in vitro* and *in vivo* with a new simple and rapid method. It measures the rate of DNA unwinding in alkali, which is a function of the size of single stranded DNA. *In vitro* X-irradiation of cells at 0°C produces a decrease in the molecular weight of DNA as a linear function of the dose. The target size of the DNA is about $3.5 \times 10_9$ daltons, and the dose for 1 break is 111 R. Repair processes are very rapid since irradiation at a slightly higher temperature resulted in a DNA with less breaks.

Work is being completed on the carcinogenic properties of inhaled direct-acting alkylating agents. Previously, studies in this laboratory had shown that dimethylcarbamoyl chloride (DMCC), epichlorohydrin (ECH), and bis(chloromethyl)ether (BCME) were potent respiratory tract carcinogens in the rat.

Histological evaluation of epichlorohydrin-exposed rats is now complete. There were 15 cases of squamous cell carcinoma of the nasal cavity out of 140 rats given 30 exposures to 100 ppm ECH. This contrasts to the results of the chronic exposure studies where the tumor incidence was 2% (one squamous cell carcinoma of the nasal cavity and one laryngeal papilloma) at 30 ppm and no cases at 10 ppm. Appropriate government agencies have been notified of these findings.

Studies with bis(chloromethyl)ether (BCME) have been expanded to include exposures of rats to mixtures of gas phase hydrogen chloride (HCl) and formaldehyde (CH_2O). It is known that BCME can be formed by a gas phase reaction of these compounds. To date, 22 out of 97 rats chronically exposed to 10 ppm HCl plus 10 ppm CH_2O have died with squamous cell carcinoma of the nasal cavity. This is the same type of tumor seen in the DMCC and ECH exposures and the high tumor yield is indicative of strong direct-acting alkylating activity.

Studies of the hematotoxicity of benzene are nearing completion. Peripheral blood cytopenic responses were elicited from Sprague-Dawley rats. AKR mice C-57 Bl mice, and CD-1 mice chronically exposed to 300 ppm benzene. Lymphocytopenia appeared in all exposed animals and, in addition, anemia appeared in all exposed mice. At autopsy, many exposed animals exhibited hypoplastic bone marrows and spleens with marked amounts of hemosiderin pigments. Studies at 100 ppm with Sprague-Dawley rats and AKR mice have demonstrated a dose response effect with respect to peripheral blood cell depressions.

Research in Radiation Dosimetry and Carcinogenesis. Research projects in radiation dosimetry and carcinogenesis can be categorized in one of four major areas, i.e., (1) mechanisms of radiation damage and its repair in tissue, (2) radiation dose response relationships, (3) biological behavior and radiation dosimetry of alpha-emitting radionuclides, and (4) new developments in methodology for measurement of radionuclides by *in vivo* gamma spectrometry and radiochemical techniques employing alpha spectrometric techniques.

Current work involves the estimation of the risk of bronchial cancer in populations exposed to natural and slightly elevated levels of radon daughters. Recent published data indicate that when both indoor and outdoor exposure to naturally occurring radon daughters is considered, the median annual alpha dose to the cells in bronchial epithelium is 150 mrad. If a quality factor of 20 is used for this radiation, the median alpha dose is 3,000 mrem per year for persons in the U.S. This is about 1/10 of the whole lung exposure allowed occupationally and is the largest dose received from any naturally occurring radionuclide.

Irradiation of rat skin with ultraviolet light produced tumors in proportion to the dose, but the tumors were almost exclusively keratoacanthomas, a type of tumor not often seen after exposure to ionizing radiation. When ultraviolet light and ionizing radiation were combined, each type of radiation produced tumors independently of the other. Thymine dimers in epidermal DNA were found to correlate well with the incidence of skin tumors for ultraviolet wavelengths in the solar spectrum and at 254 nm. When a polycyclic hydrocarbon carcinogen, 7,12 dimethylbenz(a)anthracene (DMBA), was applied to rat skin, histologically the tumors were induced as if the two agents were acting independently.

Research in Repiratory Disease in Aerosol Physiology. A major area of investigation in studies of the diagnosis of respiratory diseases is the kinetics and mechanisms of clearance of particles from the respiratory tract after deposition in the bronchial tree. Aerosols continue to be used to help define the mechanics of particle deposition and transport within the respiratory tract.

A series of investigations have been completed on particle deposition in human volunteers in vivo and in a hollow airway cast extending from the larynx to the 2mm diameter bronchial airways. In the first six generations of the cast, the deposition efficiency varied with Stokes number for aerosol AMAD's > 2 μm. In vivo alveolar and total deposition in nonsmokers was minimal at ∿ 0.4 μm, while alveolar deposition peaked at ∿ 3 μm aerodynamic diameter. Electrostatic charges at levels of 360 to 1,000 negative charges/particle significantly increased deposition in the hollow cast. Most of the enhancement in deposition was found in the larynx and in the trachea. The increase in deposition followed the theory developed by Yu of SUNY-Buffalo. Airflow and turbulence measurements were made with X-Y anemometer probes within the airways of hollow casts and "ideal" tubular models, and differences in patterns of flow.

A study of the effects of chronic inhalation exposures to sulfuric acid mist upon mucociliary clearance from the lungs has been completed. The donkey was used as an analogue for man. Four animals were exposed one hour per day, five days per week, for six months. The mean mass concentration of acid mist was 100 $\mu g/m^3$, and the mass median aerodynamic diameter was ∿ 0.5 μm. Clearance was monitored by serial, external in vivo measurements of the retention of an insoluble, radioactively-tagged, ferric oxide aerosol, which was inhaled following exposure to the acid. Bronchical clearance became erratic within the first week of exposure; rates were significantly different, usually slower than the control on many test days although the degree of response varied among the four animals. Two animals exhibited a sustained impairment of clearance towards the end of the six-month exposure period and continued to have erratic clearance during a three-month follow-up period.

A study of high community noise levels affecting millions of adults and children across the country has been initiated. The objective of this study is to measure the risk to children of developing noise-induced type hearing loss upon exposure to high noise levels predominantly from aircraft using John F. Kennedy and LaGuardia Airports. The study will utilize the records of the extensive hearing screening and testing programs of the New York City School system to locate cases of noise-induced hearing loss and suitable controls.

The third follow-up of children given X-ray therapy for ringworm of the scalp (tinea capitis) with an average follow-up time of 27 years post-irradiation is being completed. Excess brain tumors, thyroid tumors and psychiatric illness are continuing to appear in the irradiated group, as compared to the controls (who had tinea capitis but no irradiation). The excess rate of skin cancers is increasing sharply with elapsed time since irradiation. There is a clear racial effect, with no excess skin cancers appearing among blacks.

Women irradiated for acute postpartum mastitis, as compared with siblings and with women who had the disease but no radiation treatment are being studied. The study shows an excess of breast cancer beginning 10-15 years after irradiation and continuing to at least 35 years post-irradiation (the maximum length of follow-up). The dose-response curve is essentially linear up to a dose of about 400 rads, and thereafter diminishes with increasing dose. Greater dose fractionation of the radiation has no apparent affect on tumor response. The joint effects of having radiation exposure and other risk factors for breast cancer are being evaluated. The general finding is one of no synergism between radiation and other risk factors, with the possible exception of cystic breast disease.

A retrospective history of hair dye use has been obtained from women with breast cancer and a comparable group of women without breast cancer. The data permits analyses of frequency, duration and timing of hair dye use ten or more years prior to diagnosis. The relationship is seen most clearly among post-menopausal women and among those at low natural risk of breast cancer.

The evaluation of the benefits and risks associated with the detection of disease through the use of periodic screening programs is being continued. A probabilistic model for disease screening has been developed and applied to breast cancer, in particular, data from the HIP breast cancer screening project. The observed morbidity and mortality in that study are shown to be well-fitted by the predicted values obtained from the model.

Vanderbilt University

The Center at Vanderbilt, although diverse, focuses primarily on basic mechanisms of toxicity and on natural toxins.

Studies of the toxic stress metabolites from the sweet potato have resulted in the isolation of three 1,6-dioxygenated-myoporanes (6-myoporol, 1-myoporol, and myoporone) previously unreported in this plant. Myoporone is a known toxic agent in some plants of the genera Myoporum and Eremophila that has been implicated in livestock poisoning in Australia. Thus myoporone joins epingaione (ipomeamarone) and dehydroepingaione (dehydroipomeamarone) in a group of furanosesquiterpenes that are stress metabolites of the sweet potato and normal metabolites in Myoporum species. Preliminary studies on 6-myoporol show it to be a more potent hepatotoxin than the other furans found thus far in the sweet potato.

Investigations of the metabolic activation of a 4-ipomeanol by liver microsomes has led to the study of the formation of 1,4-ipomeadiol and ipomeanine which accompanies covalent binding of 4-ipomeanol to microsomal protein. It was found that mixed-function oxidase inhibitors such as SKF525A and metyrapone inhibit covalent binding but have little effect on ipomeanine and ipomeadiol formation. The results from these studies indicate that a species with radical character may be produced during microsomal metabolism of 4-ipomeanol, but the question as to whether this intermediate is responsible for the toxic effects of 4-ipomeanol remains open.

The investigation of the nature of the activated intermediate from 4-ipomeanol metabolism has reached the point where further information can be gained only by isolating adducts and determining their structure by spectroscopic means. A number of nucleophiles were screened for this purpose and although an ideal nucleophile has not been found, it appears that either cysteine or ethyl cysteinate are closest to ideal.

In metabolic studies on vinyl chloride (VC), covalent binding of ^{14}C label from VC to protein and DNA was found to be catalyzed by rat liver microsomal cytochrome P-450. The binding was not enhanced by trichloropropylene oxide, an inhibitor of the enzyme epoxide hydratase. However, experiments with this inhibitor suggest that chloroethylene oxide is a substrate for the hydratase; thus, the epoxide appears not to be the alkylating agent formed from vinyl chloride. ^{36}Cl label from vinyl chloride is released as chloride ion during activation. Phenobarbital administration induces *in vivo* covalent binding of vinyl chloride at low (10 ppm) but not high (250 ppm) concentrations; total metabolism is not induced at either level. However, *in vitro* binding and total metabolism are both induced.

Studies with antibodies prepared to highly-purified rat liver microsomal cytochromes P-450 indicate that the microsomal cytochromes P-450 of a number of extra-hepatic rat tissues are similar to the hepatic cytochromes P-450; the extra-hepatic tissues examined included the lung, kidney, spleen, brain, heart, testis, stomach, colon, and small intestine. Similarity was observed both with double-diffusion analysis and by inhibition of benzo(a)pyrene hydroxylase and 7-ethoxycoumarin *O*-deethylase activities.

Rat liver nuclei were purified and found to catalyze the covalent binding of aflatoxin B_1 to endogenous nuclear DNA. Antibodies raised to microsomal cytochromes P-450 produced similar inhibition patterns with microsomes and nuclei when either 7-ethoxycoumarin *O*-deethylase or aflatoxin B_1 binding was assayed. The immunological similarity of the nuclear and microsomal cytochromes P-450 was also demonstrated using double-diffusion analysis. The aflatoxin B_1 adduct isolated from a hydrolysate of nuclear incubation of of microsomes with calf thymus DNA. However, microsomal cytochromes P-450 appear to be responsible for most of the metabolism of aflatoxin B_1 to DNA adducts at all aflatoxin concentrations.

A method for the isolation of human syncytiotrophoblast membranes has been developed. Purified syncytiotrophoblast vesicles have been characterized biochemically, morphologically, and physiologically. Enzymatic analysis of the membrane vesicles indicated that both acetycholinesterase and choline acetyltransferase are associated with the vesicles. In addition, a specific binding site on the membrane has been identified which may be a muscarinic cholinergic receptor. Using this model, it was shown that pharmacologic levels of cholinergic ligands do interact with the placental membrane in a non-specific manner to alter membrane permeability.

Using this membrane vesicle preparation, the effects of two heavy metals on human syncytiotrophoblast membrane preparation were studied. Cadmium and mercury effect the transport of nutrients, such as amino acids across the placenta. These heavy metals induced significant placental toxicity.

2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) is teratogenic in a variety of animal species. Biotransformation and fate of 2,4,5-T in the rodent has been the subject of considerable investigation. Studies during the past year show that only 2,4,5-T and no other metabolites are found in the placentas, yolk sacs, and embryos. Since a high dose of the herbicide is reported to induce toxicity and/or teratagenicity in mice, the present study suggests that 2,4,5-T itself is associated with the high incidence of fetal resorptions and cleft palate in offspring of mice exposed to 2,4,5-T during pregnancy.

Rubratoxin β is a hepatotoxic and nephrotoxic material. Saturation of the β-lactone ring eliminated these effects. Similarly, disruption of the molecule to yield parasorbic acid also eliminated the toxicity. Rubratoxin B inhibited liver microsomal metabolism, the mechanism being similar to other agents which induce fatty liver.

During the past 12 months studies of the relationships of metal ion toxicity to other parameters in both theoretical and experimental studies have been extended. A comprehensive survey of the relationships between metal ion toxicity and the parameters used to characterize the hard/soft nature of metal ions has revealed several previously unsuspected statistical correlations among the various groups of metal ions. The correlations are of value as a guide in studies which seek to use the regularities among metal ions as a guide in the design of experiments seeking the most effective chelating agents for therapeutic testing for a given metal.

One area in which these results have been used as a guide is to examine the role of metal ion pretreatment in offsetting the lethality of subsequent injections of the same metal ion. From the preliminary data now in the literature it appeared that this might be a type of behavior common to all of the "soft" metal ions. Experimental test of this hypothesis showed that, in fact, it was not correct. Only those soft metal ions capable of forming a linear or tetrahedral coordination geometry seem capable of inducing a protective process (presumably involving enhanced metallothione in synthesis) in the animals, i.e. Cd^{2+}, Hg^{2+}, Au^{+}, Ag^{+}, Sn^{2+}, and Cu^{2+}. Those soft ions which form only square planar or octahedral complexes were not capable of providing any measure of protection against lethality subsequent to pretreatment.

A number of new chelating polymers have been synthesized according to a design which should allow the slow hydrolysis of low molecular weight chelating fragments from them as they pass through the gastrointestinal tract. These polymers all appear to have a very low level of oral toxicity, with LD_{50} values appreciably in excess of 1 g/kg. The low molecular weight fragments which have been incorporated in them include chelating agents such as cysteine, methionine, dimercaptosuccinic acid and N-acetyl penicillamine.

A study which has been carried out in part is a search for the optimum agent, among currently available therapeutic chelating agents, for the treatment of acute mercury poisoning. D-penicillamine, N-Acetyl D,L-penicillamine, 2,3-dimercaptorpropanesulfonate and 2,3-dimercaptosuccinic acid seem about equally useful when administered under conditions where the molar excess of the chelating agent is very large. Under more stringent conditions the relative sequence of these materials is approximately: dimercaptosuccinic acid > dimercaptopropanesulfanate ∿ N-Acetyl-D,L-penicillamine > D-penicillamine. When these experiments are completed we will have an effective set of standard conditions for use in comparing the effectiveness of newly synthesized chelating agents with the best ones presently available.

The utility of nude (Nu/Nu) mice as recipients of human tissue xenografts in the evaluation of potential human toxins is being investigated. Since ethical considerations create necessary but severe restrictions in the scope and magnitude of clinical research involving human objects, mammals bearing human tissue potentially susceptible to suspected chemical and/or biological toxins would provide a unique tool to the field of experimental toxicology. This approach has been made possible by the recent discovery of the mutant nude mouse and its extensive study revealing that the ability to transplant xenografts is due to its genetic lack of a thymus and the consequent failure of the cell mediated immune system. It was demonstrated that the functionality of xenografts in nude mice by successful amelioration of chemically-induced diabetes in the nude by peritoneal rat Islet transplants. A specialized pathogen-free facility has been constructed for breeding and long-term experiments involving nude mice. Breeding stocks using a Balb/c or a NIH Swiss genetic background are currently being expanded. Liver xenografts have been initiated and are being evaluated as to the method of implantation and viability.

In further studies of the metabolism of carbon disulfide (CS_2), the metabolism of CS_2 to carbonyl sulfide (COS) in vivo has been examined. The results of these experiments indicate that CS_2 is metabolized to COS in vivo. In untreated rats it is the predominant metabolite excreted in the expired air. On pretreatment with phenobarbital more of the administered CS_2 is metabolized to COS and CO_2 with CO_2 now being the predominant metabolite. This increased rate of in vivo metabolism of CS_2 in phenobarbital pretreated rats is in agreement with in vitro data showing an increased rate of metabolism of CS_2 to COS using microsomes from phenobarbital pretreated rats. The in vitro data indicate the metabolism of CS_2 to CO_2 involves first the cytochrome P-450 monooxygenase catalyzed metabolism of CS_2 to COS followed by metabolism of COS to CO_2 by these same enzyme systems. The present experiments suggest the same sequence of reactions is operative in vivo.

Studies of the factors affecting the induction of the enzyme DT-diaphorase by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) have been carried out. TCDD is a potent inducer of rat liver DT-diaphorase. The induction of DT-diaphorase activity is rapid, showing a 50-100% increase in activity in rat liver 6 hours after administration of TCDD. Prior administration of actinomycin-D prevents the increase in activity. Approximately the same degree of induction of DT-diaphorase activity (15-20 fold) is seen in adult male and adult female rats receiving their respective LD_{50} dosages of TCDD. Administration of TCDD also brings about an increase in NADPH-diaphorase activity in a number of extrahepatic tissues of the rat. In contrast to the rat, the adult male guinea pig, the species most sensitive to the toxic effects of TCDD, exhibits little or no increase in DT-diaphorase activity in various tissues in response to TCDD administration.

In previous reports the toxic nature of the mint plant, Perilla frutescens, was reported. Three principal lung toxic compounds are found in the plant; perilla ketone, egomaketone, and isoegomaketone. These three toxins have been synthesized and their pulmonary toxicity in rodents has now been well established. They are closely related chemically to lung toxins from the sweet potato. Unlike the lung toxins from the sweet potato, however, perilla toxins do not affect any other organs of the mouse.

A natural outbreak of acute bovine pulmonary emphysema (ABPE) was investigated in Cheatham County, Tennessee in which several head of prime Hereford cattle were lost as the result of consuming the dried flowering heads of dead perilla plants indigenous to a grass-deficient pasture.

Additional pathology studies using sheep, goats, ponies, and laboratory rodents revealed the marked toxicity of perilla ketone for livestock by intravenous injections, whereas intraruminal administration in goats required at least a fifteen-fold increment in dosages for a lethal response. Gross signs of perilla ketone lung toxicity were nearly identical in all animals to that seen in 4-ipomeanol intoxication. Light microscopy revealed edema of interstitial tissues along with changes in type II cells and macrophages. Electron microscopy gave further evidence of edema involving other lung cell types.

Harvard University

A significant new development involving this Center and the one at MIT is the planning of a joint MIT-Harvard program in toxicology and environmental health. The administration at MIT is eager to develop this. Harvard is traditionally less well structured for an activity of this sort, but discussions with faculty in the Arts and Sciences, the Kennedy School of Government, and the Medical School have identified substantial interests and much individual activity in various areas of toxicology, environmental science, and public policy aspects of environmental and occupational health. It is anticipated that further discussions and planning will lead to major new undertakings involving the two universities and the two NIEHS-supported centers.

Research activities summarized here are organized into three main groups - toxicology, radiobiology, and epidemiology.

The Toxicology program is divided into two groups - Toxicopathy of the Respiratory System and Biochemical Toxicology. Most of the research in the respiratory system represents an attempt to explore the diverse physiological mechanisms which prevent the accumulation and deleterious action of inhaled particles and gases. Attempts are being made to understand the factors that influence aerosol deposition, e.g., the effects of species differences, breathing patterns, and gravity. At the same time, extensive studies are being conducted to determine how non-ciliated parenchyma responds to inhaled particles on deposition. The studies also deal with the nature, migratory patterns, and behavior of pulmonary macrophages since these are often pivotal events in the pathogenesis of pulmonary disease.

The work of the Biochemical Toxicology group has been concerned with the inhalation toxicology of plastics intermediates, including particularly acrylonitrile, ethylene and its halogenated analogues, and chlorobutadiene. Studies on the hepatotoxicity of ethylene in rats pretreated with polychlorinated biphenyl show that injury is a function of metabolism of the ethylenic group to an epoxide intermediate. The toxicity of chloroprene (2-chlorobutadiene) is affected by metabolism in the opposite manner from vinyl chloride or ethylene, i.e., toxicity is increased by metabolism. In this regard, it is similar to 1,1-dichloroethylene, which is directly hepatotoxic, but less so in PCB-pretreated animals.

A pilot study was initiated on employees of a polyurethane manufacturing plant who complained of inability to urinate and of certain peripheral neuropathies. This report led to a full NIOSH medical evaluation. The pilot study, though preliminary, indicates that the catalyst used in the plant is the probable cause of the problem. This substance (NIAX^{R}Catalyst ESN) is a proprietary product of Union Carbide Corporation which causes significant bladder damage in rats administered the material by gavage. Rats injected i.p. with 2.0 ml/kg ESN died almost instantaneously (within one minute) after severe convulsions. Rats injected with 1/10 that amount showed reduced motor activity, rapid breathing, loss of aggression, and an inability to lift their heads. ESN is a nominal mixture of 3-(dimethylamino)propanenitrile (95%) and 2,2'-oxybis(N,N-dimethylethanamine) (5%). Use of ESN was discontinued after symptoms of toxicity in the workers became apparent.

The Radiobiology and Experimental Carcinogenesis group at the Harvard EHS Center is investigating the carcinogenic and mutagenic effects of radiation, which may serve as a model for the action of some chemicals at the level of DNA. A further aim is to evaluate effects of combined exposure to radiation and chemical agents. A particular focus of all of the in vitro studies is the elucidation of the role of DNA repair processes in the expression of the cellular effects of radiation.

Long-term studies are continuing using ^{210}Po as a source of localized alpha radiation in the lungs along with benzo(a)pyrene as a local chemical carcinogen and diethylnitrosamine (DEN) as a systemic carcinogen. Past tumor induction studies have focused on the influence of total dose and dose distribution on the induction of lung cancer by alpha radiation and on the interaction of alpha radiation and B(a)P.

Experiments have continued in two additional areas: (1) the role of cell proliferation in the induction of experimental lung cancer, and (2) the importance of dose-rate in induction of experimental lung cancer by alpha radiation. There is considerable evidence to suggest that the carcinogenicity of agents may be enhanced if the target cells receive an appropriate stimulus to proliferate sometime after exposure to the carcinogen.

Studies are being conducted on mammalian cells in vivo in an effort to learn more about the cellular effects of radiation. Of particular interest is the correlation of malignant transformation with mutation, survival and longevity in irradiated mammalian cells, and the role DNA repair processes play in these phenomena. Studies involve both cultured human diploid cells and rodent cells. It has been found that the tumor promoting agent 12-0-tetradecanoly-1-phorbol-13 acetate (TPA) is most effective when present throughout the proliferative phase of the expression period after irradiation. No effect was observed when the cells were incubated with TPA for only a 48-hour period after irradiation. Certain protease inhibitors were found to have an inhibitory effect on transformation after irradiation, possibly because they suppress error-prone DNA repair. These substances also affect the frequency of spontaneous and X-ray induced sister chromatid exchanges.

In the mutagenesis program at the Center, the aim is to evaluate the importance of mutations in carcinogenesis. In particular, efforts are being made to determine the extent to which mutations induced by radiation correlate with the malignant transformation of cells in vitro. Two techniques are being used. In the first, the mutation rate in cultured cells is measured by scoring resistance to 8-azoguanine (HGPRT locus) or Ouabain. In the second, sister chromatid exchanges are measured. The dose response characteristics for these endpoints are determined initially and compared with those of transformation. Post-irradiation mutation rates are scored in plateau phase cells as a function of repair time and evidence is sought for a biphasic curve such as that seen in transformation studies and thought to reflect the action of two repair processes, one error-prone and the other error-correcting. A comparison of previously published results of transformation frequencies induced by ultraviolet light with mutation frequencies for the Na/K ATPase gene locus reveals that transformation occurs about 10 times the rate of mutation at this locus at a given ultraviolet dose level. It is concluded that the transformation target is the genones controlling for plasma membrane Na/K ATPase. This system is now being used to study in parallel the effects modulation of conditions known to affect DNA repair processes in response to ultraviolet light.

Genetic susceptibility studies are being carried out to determine the susceptibility of human groups to the mutational or carcinogenic effects of radiation and other environmental agents. Over the past several years, a number of laboratories have examined X-ray survival primarily in terms of clonogenicity for a variety of cell strains derived from patients with rare diseases that predispose to malignancy. With the exception of ataxia telangiectasia (AT), reports on X-ray sensitivity have been variable. The degree of heterogeneity among normals has remained unstudied and methods used in different laboratories vary considerably.

Investigations in this Center have recently been completed to determine the range of response of some 60 strains of human diploid fibroblasts in vitro to killing by X-rays. Of the 60 strains studied, 12 were more sensitive than normal. These were derived from retinoblastoma, progeria, or AT patients. One of the strains came from a patient with Turcotte's syndrome and 2 from patients with trisomy of chromosome 13.

Strains with normal or near normal sensitivity composed the bulk of the strains. These included a number of strains from individuals with characterized genetic anomalies (Lesch-Nyhan disease, ring 13, D-deletion syndrome, Down's syndrome, etc.) or with conditions associated with a predisposition to malignancy (Fanconi's anemia, ACR, Bloom's syndrome, etc.).

Additional experiments have been carried out to determine if tumor curability in vivo by ionizing radiation is related to the intrinsic radiosensitivity of its cells and eventually the basis of sensitivity. Of nine lines studied, osteosarcoma cells, least responsive to radiotherapy, were found to be capable of repairing the most irradiation damage and to carry out the repair process for at least 24 hours after irradiation. In general, the susceptibility and resistance of the lines seemed to be related to clinical curability by radiation. Observations are being extended to include several other solid tumors as well as a group of leukemias.

Epidemiological Studies. Two types of epidemiological studies are being carried out at this Center. One involves field studies of relationships between respiratory and other diseases and various environmental and occupational pollutants. The other involves primarily the use of mortality data and work and social histories. Among the studies being pursued are those on cancer mortality and morbidity among rubber workers, mortality among Boston firefighters, 1915-1975, mortality among shoe manufacturing workers, occupational respiratory disease, occupational cancer, patterns of death in Boston by cause and possible birth defects related to parental employment in the rubber industry.

In the studies on effects of SO_x and respirable particulates on human health, a cohort of adults aged 25-74 in six communities has been established. These are being followed prospectively. At the conclusion of the first cycle of measurements, it has been found that the samples selected are close to the distribution shown in the U.S. Census for age, sex and occupation with the possible exception of one city.

Analysis of the cross-sectional data indicates that for both age and height adjusted levels of forced expiratory value and for specifically defined chronic obstructive lung disease rates, standardized for age, differences between smoking groups were apparent. Differences for these parameters between sites suggest a trend for each, which was associated with levels of pollution. Further analyses of the prospective data currently being collected will be required before definitive statements can be made about the effect of specific levels of exposure.

Mortality and morbidity from cancer among a cohort of 13,570 white male rubber workers were examined. Each man worked for at least 5 years at the Akron, Ohio, plant of the B.F. Goodrich Company. The potential period of follow-up was from January 1, 1940 to June 30, 1976. Departmental work histories were based primarily on records maintained by Local #5, United Rubber Workers. The occurrence of cancer was measured by death certificates and by a survey of Akron-area hospital tumor registries from 1964 to 1974. Two types of analyses were made: (1) an external comparison of mortality rates of rubber workers versus rates of U.S. white males, and (2) an internal comparison of cancer morbidity rates among persons who were employed in various work areas of the plant. Excess cases of specific cancers (observed/expected numbers) among workers in specific work areas included: stomach and intestine: rubber making (30/14.4); lung: tire curing (31/14.1), fuel cells and/or deicers (46/29.1); bladder: chemical plants (6/2.4), tire building (16/10.7); skin cancer: tire assembly (12/1.9); brain cancer: tire assembly (8/2.0); lymphatic cancer: tire building (8/3.2); and leukemia: calendering (8/2.2), tire curing (8/2.6), tire building (12/7.5), elevators (4/1.4), tubes (4/1.6), and rubber fabrics (4/1.1).

A common initiating event in carcinogenesis following chemical exposure is thought to be DNA damage. The frequency of sister chromatid exchanges (SCE) is a measure of DNA damage which can be detected by examining under a microscope the metaphase of peripheral lymphocytes which can be obtained by a venipuncture. Exposure to carcinogens should elevate the rates of SCE. Four pilot studies have been conducted to determine whether exposure to either suspected occupational carcinogens or carcinogens in cigarette smoke causes an elevation in the frequency of SCE's in exposed workers or cigarette smokers. Studies of individuals exposed to various carcinogens under occupational conditions are in progress but definitive results are not yet available. These studies are important in that they are among the first attempts to find and utilize ways to move from simple indicators of mutagenesis in lower organisms to real life situations in humans.

Oregon State University

The center at Oregon State University (OSU) is concerned with toxicology of both synthetic and naturally-occurring environmental chemicals.

A study has been initiated with the pyrrolizidine alkaloid (PA), monocrotaline. Rats were injected with the alkaloid (65 mg/kg). After 90 minutes they received intravenously ^{14}C-labeled palmitate and then Triton WR 1339. They were sacrificed after a further 90 minutes by exsanguination and the livers removed. The distribution of label in the lipid extracts of the serum and liver was measured. The data are not conclusive but suggest a reduction in lipid secretion in the PA-treated animals compared with controls.

In studies of lead in combination with other agents, adult male Sprague-Dawley rats were exposed to 0, 26, or 2600 ppm lead as lead acetate in their drinking water for 7 months, then placed on diets containing ethylurea and sodium nitrite (EU-N) for an additional 5 months, and observed for the development of tumors. EU-N exposure induced a high incidence of lymphosarcomas in all groups. The 2600 ppm lead group also showed a high incidence of renal tubular carcinomas which were not seen in the EU-N only or low lead plus EU-N groups. The renal tumors suggested a direct carcinogenic effect of the lead acetate. However, no conclusions regarding the interaction of lead and EU-N in the production of tumors could be drawn.

In progeny from mothers exposed during pregnancy to a combination of methylmercury chloride plus ethylurea and sodium nitrite polycythemia developed as early as one month of age in as many as 24 percent of the offspring. Many features of this condition are similar to those of polycythemia-vera in man, e.g., elevated white and red blood cell counts, splenomegaly, and hyperplasia of bone marrow accompanied by megakaryocytosis.

Mice were exposed to three different cadmium compounds (sulfate, acetate and chloride) for 10 weeks and then inoculated with encephalomyocarditis virus. Cd appeared to protect against viral-induced mortality in 8 of the 9 Cd-treated groups of mice as compared to controls. Mortality decreased as the Cd dose increased in groups of mice given either the sulfate or chloride. Cadmium acetate was more toxic than the chloride or sulfate form as noted by significantly decreased body weights. Less protection against viral infection was also noted in mice receiving Cd acetate. The latter effect may be due to a combination of Cd toxicity in the presence of an overwhelming dose of a pathogenic antigen.

Mice were exposed to nickel in the drinking water for 10 weeks and then inoculated with encephalomyocarditis virus. Doses of Ni ranged from subclinical to lethal. Virus-induced mortality in Ni treated mice, even at subclinical doses of Ni, was enhanced as compared to the control group. No histopathologic lesions were oberved in treated mice.

Dietary selenium and lead decreased the incidence of spontaneous mammary tumors in C_3H female mice. The protective effect of selenium was less pronounced in mice fed a "growth diet" that was higher in fat and protein content than the "maintenance diet" which was also used in this experiment.

Progress has been made in further characterizing the impurities in pentachlorophenol. Technical pentachlorophenol (PCP) and its salts have been widely used in agriculture and industry since 1936. The United States production of PCP exceeds 50 million pounds per year (U.S. International Trade Commission, 1974), and constitutes a major industrial product. Most of the technical PCP is used in the wood products industry for insect, fungus and slime control. Because of the chemistry involved in the manufacture of PCP, a wide range of chlorinated impurities is usually present at levels ranging up to 15 percent. These impurities include chlorinated dibenzo-p-dioxins, dibenzofurans, diphenyl ethers, hydroxydiphenyl ethers, hydroxybiphenyls and higher homologs or oligomers of these compound classes. The major (1.7%) impurities are the three hydroxynonachlorodiphenyl ethers ($HO-Cl_9-DPEs$). During the past year, $2-HO-Cl_9-DPE$ (predioxin), $3-HO-Cl_9DPE$ (isopredioxin 1), and $4-HO-Cl_9-DPE$ (isopredioxin 2), each in high purity (>99%), were isolated from technical PCP by high pressure liquid chromatography, with the ultimate objective of determining their toxicity and biochemical effects.

Photodecomposition of aldicarb was studied. In dilute solution aldicarb produces dimethyl disulfide, α-methylthioisobutyronitrile, carbon dioxide, and methylamine. Similar photodecomposition of methomyl generates a mixture of products, which includes methyl acetate, acetonitrile, dimethyl sulfide, dimethyl trisulfide, and acetone oxime. An intramolecular hydrogen atom abstraction followed by N-O cleavage in competition with S-C homolysis is viewed as a reasonable hypothesis for aldicarb photodecomposition. The methomyl photodecomposition also involved N-O bond cleavage, even though intra-molecular hydrogen abstraction is not possible.

X-ray diffraction studies of brominated fatty acids and brominated lipids were continued. Monolayer studies demonstrate that when bromine is present the fatty acid chains are in a fluid phase. Langmuir-Blodgett multilayers have been successfully produced with a dipping apparatus and studied by X-ray diffraction. Oriented lamellar samples of dipalmitoyl lecithin and its corresponding monobrominated analog have been studied by X-ray diffraction, and the presence of bromine is readily apparent in the diffraction patterns. The thermotropic behavior of the brominated analog differs significantly from that of dipalmitoyl lecithin. The technique of X-ray diffraction in conjunction with modern electronic data acquisition methods, has been shown to provide rapid, useful information on the effects of chemicals on ordered systems of biological relevance.

In efforts to develop statistical methodology for estimation of parameters in pharmacokinetic models, a comparison was made of five procedures for estimating the mean elimination rate of a population of animals modeled as one-compartment systems. The procedure based on the principle of maximum likelihood provides the best point estimates and also seems promising for the construction of interval estimates.

Research efforts begun several years ago in the area of the statistical methodology of parameter estimation in compartmental models have been continued. Procedures for a simple one-compartment open model with a stochastic drug loss parameter have been investigated. The exact and approximate densities obtained yield exact and approximate likelihood functions. Using numerical function minimization (maximization) procedures on all likelihood functions yields point MLEs. No concrete joint confidence hyper surface work has yet been done on this model to the point where it can be reported on. The joint confidence hyper surface work is in progress. Realistic data for the drug peridione (an anticonvulsant for epileptics) was generated via standard simulation procedures. This data is used in the exact and approximate likelihood functions to yield the MLEs. The Wilcoxon rank sum test was used (even though the sample sizes are small, N = 10) to test the different methods for significance. The three term Gram-Charlier based likelihood function and the log gamma based likelihood function seem to be the more desired methods to date.

In attempts to estimate growth parameters in a linear model with correlated errors, a procedure presented by G. O. Zerbe (1977) was used to compare one set of growth curves with another. In addition an attempt was made to develop a test of significance of the difference between two sets of curves. The comparison procedure involves computing a measure of the distance between two growth curves over an interval of time. The significance test is based on interval estimation procedures. Both the comparison and the test were applied to a selection of rat data used in the previous studies. Conclusions from these analyses were similar to those obtained under the analysis of variance procedures.

Several new studies were undertaken in the past year. One is concerned with the mechanism of toxicity of halogenated biophenyls. In this, the human erythrocyte membrane has been used as a model for the effects of halogenated antibacterials on biological membranes. Both triclocarban (TCC) and tricloflucarban (TFC) are potent hemolytic agents. However, the kinetic response of the erythrocytes, both in terms of hemolysis and cation efflux, are markedly dissimilar. The two hour hemolytic response curve for TFC is sigmoidal, as observed previously with other halogenated antibacterials, with 50% hemolysis (C_{50}) concentration of 24 μM. TCC, however, exhibits an atypical pseudolinear dose-response curve with C_{50} of about 57 μM. In addition, K^+ and hemoglobin release occur virtually simultaneously and reach a plateau level within 10 minutes after exposure to a given TCC concentration. With TFC, on the other hand, K^+ efflux precedes hemolysis as previously seen with HCP and both occur in a delayed fashion exhibiting a concentration-dependent rate of release rather than a concentration-dependent extent of hemolysis as is the case with TCC. An explanation for these differences has not yet been found but the differential response may be related to the greater susceptibility of certain cells in the population to TCC induced hemolysis.

Preliminary feeding experiments are currently underway to determine the availability of Cd to rats from crops grown on soil enriched with the metal. Studies on the biosynthesis and characterization of metallothionein in rats ingesting small amounts of ^{109}Cd in the diet are continuing.

Although the acute toxicity of Cd^{+2} has received considerable attention, little is known about the consequences of long-term human exposure to low concentrations of the metal. Primarily on the basis of experiments in laboratory animals and limited human epidemiologic evidence, hypertension and cardiovascular disease in man has been tentatively linked to prolonged exposure to low concentrations of Cd^{+2}.

Approximately 100 million tons of municipal sewage sludge are produced annually in this country, representing a major solid waste disposal problem. Increasingly sewage sludge is used as a soil conditioner and a source of nutrients for agricultural crops. Since many edible plants can accumulate high concentrations of metals such as Cd^{+2} from sludge treated soils, there is an increasing potential for high concentrations of cadmium to enter human food supplies.

A program has been initiated to further define the metabolic disposition of hydrazine. The objective for the first year of activity is to establish methods for collecting and measuring $^{15}N_2$ originating from animals given ^{15}N-hydrazine and to examine aspects of the disposition of intact hydrazine in rats. This research is designed to establish capability in the Center for more detailed study of the fate of other nitrogenous toxicants, such as nitrosamines and carbamates.

University of Rochester

The EHS Center in Rochester is divided into six research programs. In the program on dose-response and pharmacokinetics in man, four populations exposed to methylmercury in Canada, Iraq, Peru and Samoa are now under study. The Canadian population consists of Ojibway Indians in Northwestern

Ontario and Crees in Northwestern Quebec living in their Reserves. Members of these populations consume large amounts of freshwater fish, some of which have high concentrations of methylmercury. In contaminated areas of Northwestern Ontario, average concentrations in game fish are in the range of 3-5 ppm. In Quebec, mercury in fish is usually between 1 and 2 ppm. Some individuals have been found to have blood levels up to 600 ppb. Exposure is seasonal and repeats on a yearly cycle.

The outbreak of methylmercury poisoning in Iraq led to over 6,000 hospital admissions of severe cases of poisoning and to over 400 deaths. The population is characterized by a brief period of intake of mercury (2-3 months) involving all ages, both sexes, and doses up to those causing death. During the past two years hair samples have been collected from several thousand people in order to quantify their exposure history.

Some fishermen and their families living in coastal villages in Peru have blood levels of methylmercury close to those associated with signs and symptoms in Iraq and in other epidemics. This population is characterized by a lifetime of exposure to methylmercury from the consumption of large oceanic fish. Dose-response studies in this population will help determine the effect of lifetime exposure as compared to brief exposures in Iraq.

Korean contract fishermen working in tuna boats based in American Samoa have unusually large intakes of fish. Most of their time is spent at sea and most of them are employed for a period of up to 5 or 6 years. This population will be studied with particular emphasis on the relationship between the daily intake of methylmercury and steady state blood levels and hair levels. This study will be coordinated with observations on volunteers so as to determine the best method of measuring dietary intake.

The studies on exposed populations will be augmented by observations on volunteers using tracer doses of heavy metals. Studies are in progress using methylmercury labelled with the ^{203}Hg isotopes. Studies are also planned using diets of tunafish as a source of methylmercury.

Six healthy, adult male volunteers have been administered methylmercury-203 intravenously. The analyses to date indicate that the majority of the administered dose is metabolized to an inorganic form and excreted in the feces, with additional excretion in the urine. There are short-term phenomena observable in the blood curves and the blood levels fall more rapidly than the whole body levels, indicating a potential sequestration and slow elimination of some of the mercury.

The behavioral toxicology program is designed to study the toxicity of contaminants (particularly heavy metals) as manifested by changes in behavior and other functional capabilities. The rationale for these studies is that many poisons act on the central nervous system, while others reveal their earliest and most subtle consequences as behavioral changes.

Studies are underway to examine the visual changes produced by methylmercury poisoning in macaque monkeys. Eventually comparisons will be made between the precisely specified defects in visual function and histopathological data. Other studies involve vibratory sensitivity and the effects of lead and carbon disulfide on complex schedule-controlled behavior.

Work is in progress on behavioral mechanisms of drug action. In these studies emphasis is upon behavioral indicators of low-level drug and chemical toxicity, reflecting a longstanding interest in drug-behavior interactions. Some experiments address questions of stimulus control by seeking to learn more about environmental variables that modify drug action. Others concern motor output by exploring how chronic dosing with psychotherapeutic drugs (e.g., haloperidol) produce disorders of movement.

Other studies involve drug self-administration by inhalation. Monkeys are taught to press buttons in order to inhale agents such as nitrous oxide and toluene. Systematic studies of the reinforcing properties, with emphasis on the role of duration of inhalant delivery and concentration, are underway.

Visual manifestations of toxicity are being studied. A variety of agents including carbon monoxide, methanol, toluene and carbon disulfide are being tested.

The inhalation toxicology group is concerned with pharmacokinetics. Animal studies and contemplated studies in man are designed around the use of Pb-203. This radionuclide has been under investigation in this laboratory for two years, first as a feasibility study to ascertain if local (linear accelerator) production was practical, and later, when a commercial source developed, as a detailed study of chemical and radionuclide contamination and purification. This has led to a reliable purification procedure for a by-product, unprocessed Pb-203 from a commercial source.

Pulmonary clearance is being studied in experimental animals focusing on the role of the alveolar macrophage in the mechanisms of clearance functions and on the influence of various environmental contaminants on clearance. TiO_2, which has established reproducible retention characteristics, appears to depend primarily on phagocytosis for removal from the lung parenchyma. This material therefore is used, under a standardized aerosol procedure, as a marker of normal macrophage function. Kinetic and morphological evidence indicate that clearance slows down after the first days postexposure. A stimulation of the reticuloendothelial system with a resulting increase in alveolar macrophages sometimes leads to higher lung clearance but in general clearance is not very effective.

Studies are being conducted in genetic, reproductive and developmental toxicology. Heavy metals play important roles in early mammalian development. Deficiencies as well as excesses of essential trace metals can produce developmental malformation. Some non-essential metals are highly embryotoxic and some have been shown to be teratogens in mammals.

A mouse model system is being used to simulate human prenatal and postnatal exposure to methylmercury, other mercury compounds, lead, and other heavy metals with the expectation that such studies will aid significantly in examining and interpreting less extensive human data; this, in turn will aid in evaluting hazards to exposed human populations. Current and projected methylmercury studies include: (a) maternal-fetal methylmercury exchange and relative distribution of methylmercury in different maternal and fetal organs, especially developing fetal brain; (b) mother-to-newborn transmission of methylmercury via milk during the suckling period; (c) ontogenetic changes in methylmercury excretion. Pharmacokinetic compartmental analysis of perinatally exposed mice have also been initiated. The goal of this effort is to establish a developmental pharmacokinetic model of maternal-fetal and maternal-newborn transmission of methylmercury, other forms of mercury and other heavy metals. This model should allow predictive comparison of less extensive human data such as prenatal and postnatal methylmercury in Iraq and elsewhere, and will provide a basis for designing efficient studies in man in non-human primates.

In addition, assays are being developed for assessing genetic effects of environmental agents in mouse and man. Cytogenetic assays for detecting abnormalities of chromosome number in mouse oocytes and early embryos have been developed and a human _in vivo_ somatic cell mutation assay for detecting codon-specific hemoglobin amino acid substitutions is being evaluated using human blood lysates of individuals exposed to toxic environmental agents.

Large developmental differences in rates of excretion of mercury have been found. Suckling mouse pups given single non-toxic pulse doses of methylmercury _per os_ excrete very little mercury until approximately the sixteenth day after birth, when they abruptly begin excreting it at adult rates.

In the course of a recent series of studies designed to define variables which influence mercury excretion, it has been observed that dietary components have very significant effects on rates of mercury excretion. Adult animals fed a milk diet excreted mercury much more slowly than animals fed the usual pellet diet. Animals fed a defined diet with a very low fat content excreted mercury much more rapidly. This unexpected observation suggests that dietary factors may have considerable significance in influencing body burden and critical organ concentrations in human populations exposed to methylmercury.

A codon-specific somatic cell mutation assay model system is being developed, using human erythrocytes and anti-sera which will distinguish human hemoglobins differing by a single amino acid residue. A quantitative radioimmunoassay for hemoglobins has been developed which is highly specific and sensitive. This is being used to determine whether there are detectable quantities of mutant hemoglobins in bloods of AA individuals exposed to toxic environmental agents and whether these individuals differ significantly from unexposed populations.

A number of studies are concerned with metal interactions. The metabolism of orally administered radioactive cadmium was studied in male volunteers.

It was found that on the average 2.6% of the ingested cadmium was absorbed. The absorbed cadmium was excreted from the body with a half-time of about a year. In the liver the cadmium was retained longer than other tissues and the retention half-time was estimated to be between 450-650 days. The kidneys showed continuous accumulation of the isotopic cadmium and in one case the amount was doubled in 283 days. The absorption and retention half-time values obtained from this study are markedly less than those reported in the literature.

The metabolic pathway by which selenite is transformed into the chemical species which interacts with and detoxifies inorganic mercury and cadmium has been identified. The initial reaction is an apparently nonenzymatic reduction by glutathione to a selenotrisulfide. This compound is further reduced by glutathione reductase to hydrogen selenide which is the form involved in the interaction with the divalent metals. The glutathione reductase step can be inhibited by chromate. This reaction scheme has been documented for red cells which have a very high glutathione concentration. The hydrogen selenide is released from the red cells and forms complexes with plasma proteins with an atomic ratio of selenium and metal of one. Similar complexes are formed in a number of organs and also contribute to the protective effect.

To investigate the role of the cadmium-binding protein in the toxicity of cadmium, a study was carried out in rats repeatedly injected with $CdCl_2$. Metallothionein was detected in plasma of these rats by gel filtration chromatography, after 4 weeks of cadmium injection. At this time metallothionein was not detected in urine. The animals developed proteinuria and glucosuria after 10 weeks of Cd treatment and their kidneys showed signs of tubular necrosis. The excretion of cadmium in urine increased markedly. Upon gel filtration of the urine it was discovered that the cadmium was being excreted bound to metallothionein. This study revealed that the metallothionein appears in the plasma of the cadmium intoxicated animals long before the onset of renal damage and that it may be used as a screening tool in predicting Cd toxicity.

Studies by the Biochemical and Physiological Toxicology group have shown that pathological signs of damage appear in a predictable way in a brain that has taken up a sufficient amount of methylmercury, but the mechanisms that lead from the dose to the toxic effect are still unknown. The known target cells for methylmercury damage in the brain have been identified on the basis of dose-effect relations _in vivo_. The corresponding subcellular and molecular targets could not be identified simply by extending this method to concentration-effect relations _in vitro_, because of interference by endogenous, sulfhydryl-bearing ligands, which competed for mercurials. The interference varied from preparation to preparation, leaving no common measure of effective concentrations.

Processes investigated in this program included neurohumoral postsynaptic reception, the active transport of ions ("calcium pump" and "sodium pump") and protein synthesis.

The ionophoric site of a purified "calcium pump" was shown to be differentially susceptible to inhibition by mercuric ions, and resistant to merthylmecuric ions. The catalytic site, on the other hand, was susceptible to inhibition by both mercurials. This transport system was derived from the sarcoplasmic reticulum with techniques which are directly applicable to the corresponding transport system in cerebral microsomes. Parallel work on the "calcium pump" of mitochondria led to the isolation and characterization of calciphorin, the first low-molecular weight polypeptide known to function as a physiological ion carrier. Calciphorin will be of special interest as a potential target molecule in lead poisoning.

University of Cincinnati

Several new studies were initiated during the year under review and include: Factors controlling Cd absorption from the intestine, response of lymphocytes to tumor-promoting agents, in vivo assessment of neurotoxicity as revealed in neostriatal function, effects of organotin compounds on human behavioral patterns, hazards of waste water exposure arising from industrial contaminants, biotransformation of polycyclic aromatic hydrocarbons by plants, and health effects related to fiber length of environmental asbestos.

Studies of the metabolism and toxicity of cadmium continue. In a new study the role of Cd-metallothionein (CdMT) in Cd transport was further tested by determining distribution of the compound when infused into rats at rates similar to those which might be encountered under normal conditions of exposure. The preferential uptake of the compound by the kidney under these conditions is fully compatible with its suggested role in transfer of Cd from liver to kidney. The role of CdMT in urinary Cd excretion remains questionable, however, in light of the very high reabsorptive capacity of the renal tubules. The reabsorption is mediated by a mechanism which appears to be specific for certain proteins only; in particular, myoglobin inhibits CdMT reabsorption by what is probably a competitive interaction.

Progress was made in the attempts to elucidate the mechanisms controlling Cd uptake from the intestinal lumen. It was shown that the first step in this process, the uptake from the lumen into the intestinal tissue, is mediated by a saturable system near or in the brush border; release of Cd into the body is about 30 times slower than this first step. Attempts are now being made to localize Cd in the tissue by radioautographic techniques. The role of CdMT, if any, in intestinal Cd absorption is also under study.

A new project attempts to explore the early stages of acute renal failure following administration of $HgCl_2$. Uptake of Hg by the kidney, and its subsequent ability to affect renal function, appear in some way to be related to the levels of glutathione in the tissue. Involvement of SH groups in the early response of the kidney to Hg is also suggested by the observation that the reducing agent dithiothreitol can counteract many of the acute effects of the metal.

Progress was made in a toxicodynamic study of lead in rats. This species has proven to be a useful model in which to reproduce, for instance, the decreasing incremental rise in blood Pb reported to occur in humans with increasing exposure. The non-linearity of blood and renal lead with increasing exposure raises the interesting possibility that tolerance to lead can develop under specific circumstances. Associated with the development of a compartmental model of lead distribution is an analysis of the significance of the selective disposition of the metal during chronic exposure.

New information has been developed on the role of minerals in lipid metabolism. An earlier finding that Zn and Cu can reduce fatty infiltration of the liver and also hypercholesteremia in rats fed a semi-purified diet supplemented with cholesterol has now been extended to other minerals present in normal rat chow. Full supplementation of semi-purified diet with all the minerals of the chow reduced the hyperlipidemia of the fatty infiltration of the liver seen in control animals. In addition, an increased incorporation of cholesterol into high-density lipoprotein fractions was noted, a factor of significance in vascular disease. Both dietary fat and cholesterol affect absorption and catabolism of exogenous cholesterol, as shown in the peak time of label in plasma, the half-life of cholesterol in various lipoprotein fractions, and the rate of excretion of label in the bile.

A good example of the association between toxic effects and alteration in trace metal metabolism is the finding that exposure of rats to Cd in utero leads to decreased concentrations of Fe and Cu in the pups at birth. Another instance of apparent interaction between toxic and essential trace metals is the previously described effect of Zn supplementation during chelation therapy for Pb poisoning. Lead reduces the activity of the Zn-requiring enzyme ALA-dehydrogenase in blood and chelation causes further loss of Zn. A return to normal enzyme activity levels can only be achieved with Zn supplementation during chelation. A more conclusive assessment of the combination of Zn and EDTA therapy in lead intoxication is in progress.

The pulmonary and cytotoxic effects of stack exhaust condensates from coal-burning power plants are under study, as well as the somatic and behavioral sequelae of exposure to exhaust from internal combustion engines continue under investigation.

In an extension of earlier work on the introduction of various metals into the environment from coal-fired power plants, the emission of Ni and Se into the atmosphere was determined. A previously reported mass balance study accounted for 94.8% of the As introduced into the plant. Similar values have now been obtained for Se (98.2%) and Ni (92.8%). Mean amounts of the three metals released into the atmosphere were 600 g/day for As, 1840 for Se and 6600 for Ni. In contrast, emission control devices removed essentially all Al from stack effluents.

The metal content of stack exhaust condensates from power plants correlates well with toxic effects of intratracheal instillation of these condensates. The low content of organic compounds in some of the condensate samples further points to the likelihood that a major portion of the total toxicity of these samples is related to their metal content. Instillation caused damage to lysosomes in pulmonary macrophages; increased levels of lysozyme were found in lung lavage fluid. In addition, evidence was obtained for the formation of alveolar exudate rich in plasma protein. After instillation, most of the Fe^{3+} and Al^{3+} remained in the lungs; these were also the metals with the greatest toxicity to macrophages. All these samples were studied also for their cytotoxic effects in vitro, using mammalian cell lines, and for their mutagenic potential in the Ames test. There is no doubt about the presence of highly cytotoxic compounds in the condensates; evidence for mutagenicity is less convincing.

National concern about occupational and consumer exposure to carcinogens has stimulated the petroleum industry to examine crude oils and refined products by mouse bioassay and other methods. Of particular interest are the results of mouse bioassay studies of three raw oil shale, four crude retort oil and three spent shale samples. The crude oils were from four different retort processes. With repeated topical application, all of the oils were found to be carcinogenic for mice. The carcinogenicity of these oils were comparable to that of 0.15% B(a)P. None of the raw shales or spent shales were found to be carcinogenic by this bioassay method.

Bacterial cell systems such as in the Ames test were used to determine the influence of PCBs (polychlorinated biphenyls) on the induction of hepatic microsome activity in exposed rats. The same test procedure was further employed to study the formation of mutagens from benzo(a)pyrene [B(a)P] in the perfused rabbit lung. The metabolism of B(a)P in this isolated organ has been previously described; more recent experiments focused on the effects of particulate matter, SO_2 and cocarcinogens such as n-dodecane on the metabolic pattern of B(a)P. Metabolism of other pulmonary carcinogens including N-heterocyclic compounds is also under study. Another program explores the biotransformation of polycyclic aromatic hydrocarbons in plants, a potential source of carcinogens in the human food chain. Search for, and identification of possible mutagens in Cincinnati water supply also remains under active investigation.

Inhalation studies with vinyl chloride monomer in guinea pigs are continuing. Variables under investigation include vitamin C and ethanol in the diet. In preliminary experiments, promotion by ethanol of the carcinogenicity of vinyl chloride has been observed. Research also continues on the carcinogenicity of topically applied complex mixtures of importance to the nation's interest in alternative fuel sources.

Teratological problems constitute an expanding area of research. In one study in which female rats were exposed to dichloromethane before and/or during pregnancy, no significant gross anomalies were observed in the offsprings. However, significant behavioral deficits could be demonstrated shortly after birth. These deficits persisted into adult life and the effects could still be seen five months after exposure to the solvent in utero. This is another example of teratogenic effects which are demonstrated by behavioral changes, not structural defects. In similar studies in which rats were exposed to trichloroethylene by inhalation, concentrations of 1800 ppm failed to produce maternal intoxication, toxic effect on the fetus or structural birth defects.

Research continues on the effects of the herbicide TOK (dichlorophenyl-p-nitrophenyl ether) on maturation of rat lungs. The hypothesis that TOK exerts its teratogenic effect on the lung by directly interfering with synthesis of pulmonary surfactant could not be substantiated. This conclusion is based on the observation that respiratory distress of the newborn is caused by exposure to TOK only at a stage of gestation preceding by several days that at which surfactant synthesis is initiated. Presumably the action of TOK involves induction of structural malformations in the developing lung rudiment.

The teratogenicity of Cd in mouse embryo limb bud culture and its reversibility with zinc is under study. Concentrations of 10 to 20 μg/ml cadmium acetate produced abnormal cartilaginous skeletons such as limb hypoplasia, club foot and absence of forepaw. Co-administration of equimolar zinc resulted in partial protection against the teratologic effects of cadmium.

The scope of research in behavioral and neurotoxicity ranges from direct effects of toxic substances on neuronal activity to the search for subtle alterations in behavior of man or animals resulting from exposure to various substances. An investigation was begun during the past year into new methods for assessing neurotoxicity in vivo. One of these methods relates to the function of the neostriatum. After unilateral lesioning, the action of this tissue is expressed in the posture and directional rotation of the animal. The effect of drugs on this behavior may reflect changes in metabolism or neurotransmitters, and also may provide some insight into the integrity of neostriatal function. Particular emphasis centers on spontaneous and drug-induced rotation of rats exposed neonatally or chronically to Pb.

Several projects focus on behavioral and neurotoxic effects of exposure of rats to cadmium in utero. No changes could be observed in conduction velocity of peripheral nerves, or in somatosensory evoked responses in rat pups aged 7-10 days. On the other hand the latency of visual evoked responses was increased, as measured with or without anesthesia. The experimental design thus far has not been able to exclude the possibility that such effects may be related to undernutrition rather than to a direct effect of cadmium. Spontaneous locomotor activity was also affected, but here again the variables of weight and growth rate could not be completely controlled. Even though exposure to Cd in utero did not alter the birthweight of the pups, their subsequent growth rate was diminished.

The behavioral effects of cadmium exposure in utero can still be discerned in adult animals. Deficiences were found in the acquisition of reversal phase of a simultaneous visual discrimination task. By the time these measurements were taken, the abnormalities in which body concentrations of Fe, Zn and Cu observed at birth had disappeared.

Another neurobehavioral study investigated effects of perinatal exposure of rats to n-hexane; chronic exposure to this solvent is known to cause peripheral and central neuropathies in animals and man. However, no effects of exposure in utero to concentrations commonly encountered in the environment or at the workplace were observed.

Sensory irritability and psychomotor effects of hydrocarbon solvents were also investigated using human volunteers. These were exposed to Stoddard solvent and to mixed xylenes. No psychomotor changes could be discerned at levels of the solvents which are clearly capable of increasing the incidence of eye irritation.

The Division of Clinical Studies has been and is involved in a number of comprehensive epidemiological and clinical surveys of industrial populations. A comprehensive epidemiological study of styrene workers has just been completed. A detailed industrial hygiene survey in a plant involved in manufacturing fiber-glass boats showed that even with OSHA standards for the time-weighted average permissible concentrations of styrene monomer in air, intermittent high exposures led to a variety of clinical symptoms. Measurements of styrene in blood and breath and of urinary metabolites of sytrene before and after the use of protective equipment led to the conclusion that styrene is absorbed primarily from the respiratory tract and that absorption through the skin is insignificant. No neurological sequelae of exposure were noted. Statistically significant correlations were observed between exposure and worsened responses to several psychomotor tests.

An environmental and clinical study of rubber workers employed in a large rubber tire fabrication plant is underway. The objective is to determine the level of occurrence and nature of the excessive incidence of skin disease in this plant. The study encompasses identifying (1) processes, materials and types of exposure, (2) the character and types of skin disease, and (3) causal and associated enhancing factors responsible for the work related diseases.

An active clinical research program continues on the elevation of health risks associated with lead exposure in industrial populations. In one such group, whose exposure was being terminated because of a reduction in the work force, typical nuclear inclusion bodies were observed in proximal tubular cells shed into the urine. An examination of the association between blood lead levels and the BUN suggests that the correlation is best if the maximum concentration of lead is considered, rather than the product of average lead levels and years of exposure. The urea levels in blood showed better correlation with blood lead levels than with those of free erythrocyte protoporphyrin. At present a much larger population is being studied in order to confirm and extend these findings. Another interesting opportunity arose to study these relationships when it became possible to evaluate workers at a primary lead smelter before and after their exposure had been interrupted during a strike of 10 weeks duration. Preliminary findings include an apparent continued fall in porphyrins over a 10 week period following resumption of exposure, in spite of the fact that lead levels were again on the increase.

Continuing research efforts on pulmonary problems of environmental origin mechanisms responsible for disease severity in bronchial asthma and the pathogenesis of occupationally-induced isocyanate reactions. This study is being conducted in laboratory animals and in diisocyanate exposed workers.

The division of Epidemiology and Biostatistics is collaborating in a study of workers in an aluminum smelter plant. Previous work had indicated that pot room populations may be at an increased risk of leukemia, lymphomas and accidental death. Work histories from one plant in Louisiana are now being coded and the mortality follow-up is being extended over the last five years. This work forms part of a study involving cooperation between industry, labor and government. In another study the mortality of workers exposed to Ni is under investigation. Cancer of lung and nasopharynx have previously been found in such populations, and a cohort of workers has now been identified for future studies.

One of the new actively growing resources in the Center is the Aerosol Research Laboratory. It is concerned with problems of particle sizing of atmospheric pollutants and their effect on biological systems. Significant advances can be reported in the design and the construction of a sizing apparatus built on the principle of opposing jets. Some difficulties were encountered relating to the so-called edgetone effect, causing the setting up of transverse oscillations with subsequent smearing of particle separation.

Another activity involves the seroepidemiologic study of sewage treatment workers from three cities. This has now been extended into a broader study of potential hazards arising from exposure to wastewater and sludge. Such exposure may occur during collection and treatment of the wastes, as well as during the ultimate application of sludges to agricultural and other lands. Among the hazards are not only the biological ones previously studied, but also those related to the presence of toxic organic compounds in the waste water. Significant differences in antibody titers between different groups of workers have been noted. The concentration of viruses in various wastes, however, seems to be lower than previously suspected, so that there is probably no need to establish buffer zones around waste-water treatment plants for protection of the public. Work underway now has been extended from viruses to consider also bacteria, parasites and pathogenic fungi.

Massachusetts Institute of Technology

The studies at the MIT Center concern organic particulates from vaporizable fuels and particulates from coal and heavy liquid fuels. The nature of effluent particulates is significantly modified both by the nature of the fuel and by combustion conditions. In studies on the influence of type of burner and burning conditions, it was found that for vaporizable fuels a toroidal configuration of a well stirred reactor (WSR) may be of significant potential for improved mixing of fuel and air over spherical and hemispherical devices. Parallel experimental and theoretical studies are addressing issues of mixedness, measurements of residence time distributions in high temperareacting flows, cold flow simulation of the toroidal WSR and the implications of imperfect mixing on interpretation of the WSR data.

The combustion of pulverized coal particles in a laboratory furnace under conditions of interest in industrial combustors was found to produce an amount of soot and polycyclic aromatic hydrocarbons (PAH) which decreases very strongly with an increase in the concentration of oxygen in the gas in which the particles are suspended. The extent of this important effect depends upon temperature, and the composition of the PAH depends upon both temperature and oxygen concentration. The preliminary experiments suggest that the first steps of coal combustion, including particle heating and devolatilization, are probably critical in soot and PAH formation. These substances may be produced or essentially avoided depending upon the manner in which the coal is heated and contacted with air.

The organic compounds produced by the various combustion systems are being identified and quantitated by gas chromatographic mass spectrometry. Compounds already identified include PAHs and polyheterocyclics containing sulfur and nitrogen. Preliminary experiments indicate that charge exchange-chemical ionization mass spectrometry is a more powerful tool for differentiating PAH isomers than conventional gas chromatographic mass spectrometry. This technique combined with high resolution gas chromatography using both non-polar and liquid-crystal liquid phases will provide still more powerful methods for analysis of complex PAH mixtures.

Efforts are in progress to develop and test optical diagnostic systems for PAHs - specifically, to use laser-induced fluorescence excitation spectroscopy (LIFES) for quantitative detection of these substances both in flames and in exhaust streams. A survey has been conducted of the available data on the spectroscopic properties of airborne PAH to provide a data base for the analytical technique. The equipment required for this has been assembled and tested. Laboratory studies of LIFES of PAH species under isolated molecule conditions and under simulated atmospheric conditions have been initiated. In-flame studies are also in progress with pure compounds and PAH mixtures to determine the feasibility of this type of analysis.

Studies on the mutagenicity of soot and soot components indicate that polycyclic aza-arenes are generally more active mutagens than their parent hydrocarbons. There are reports of carcinogenicity of these compounds and their occurrence in particulates from combustion of fossil fuels. Studies are in progress to confirm these reports.

A number of mutagenic polycyclic aromatic compounds have been detected and identified in soot. Of particular interest are perylene, which is 6X as active as benzo(a)pyrene in the S. typhimurium test, and benzo(f)quinoline (B(f)Q), about 30% as active as B(a)P. The fact that B(f)Q is a nitrogen-containing polycyclic aromatic compound makes it of particular interest since little is known of the metabolism and biological activity of azaromatics though their chemistry has been studied extensively.

B(f)Q has been shown to bind in vitro to DNA when metabolized by NADPH-dependent microsomal systems from rat liver. Binding appears to be covalent and, by analogy to other covalent binding substances, it is suspected that B(f)Q will prove to be a carcinogen. Similar studies are anticipated on perylene.

In studies undertaken in new areas during the past year it was found that acridines bind to DNA of the P22 bacteriophage of *S. typhimurium* and are lethal in sunlight. However, photoinactivation was due to protein bound to the DNA complex. This is a route of toxicity different from DNA damage leading to mutation or neoplasm. Analysis of inactivation versus light intensity indicates that one photon is sufficient for inactivation.

Two studies have been initiated at this Center dealing with the biological impact and health effects of diesel exhaust. The aims are to construct a basic facility for collecting and analyzing particulate matter from the exhaust of a diesel engine and to determine the association between diesel exhaust exposure and the development of cancer. For the latter, a retrospective cohort analysis of approximately 25,000 former railroad employees with more than 10 years service has been established. The potential importance of this project is in its comprehensive assessment of causes of mortality in an occupation with moderate exposures to diesel exhaust using a retrospective follow-up experimental design.

RESEARCH HIGHLIGHTS

Regular Research Grants Program

The Regular Research Grants Program, including the RCDA and YES Awards, can be assigned to program areas in the approximate percentages shown: mutagenesis and reproductive toxicology 17 percent; etiology 26 percent; pharmacology-toxicology 48 percent; and pathogenesis 9 percent. Again, the percentage distribution of the major program areas has not appreciably changed over the preceding years.

MUTAGENESIS AND CARCINOGENESIS TEST DEVELOPMENT

During the past decade, a wide variety of chemicals in the environment have been shown in laboratory studies to possess the potential for mutagenic activity. Recent development and short-term tests for mutagenicity have aided in this type of research and have also demonstrated the high level of correlation between carcinogenic and mutagenic activity. The implication of these studies to man lies in the potential for carcinogenic risks and points to the need for extending the original data, and attempts for extrapolating the information to man in terms of cancer, genetic disease and birth defects. The NIEHS supports a variety of studies in the area of mutagenesis and carcinogenesis, including many facets of the problems involved in the development of new, quick, effective, and reliable test systems for mutagenicity. Among the damaging effects of a variety of environmental agents of interest to man are: abnormal chromosome numbers, inhibition of DNA repair, chromosome rearrangement, initiation of unscheduled DNA synthesis and mitotic recombinations in gene conversions. Tests for point mutations in species of Drosophila have been widely employed and studies are still underway to determine the extent of the utility of Drosophila for detection of environmental mutagens. Previous studies have shown that vinyl chloride is mutagenic in Drosophila. Vinylbromide was also found to be active in the recessive lethal tests. Out of four putative metabolites of vinyl chloride, chloractic acid and 2-chloroethanol were ineffective in the tests. Acid nitrosation of amines and amides producing nitroso-compounds under in vivo conditions may release strong pre-carcinogens (mutagens). Using carbaryl and NO carbaryl and N-methyl urea at acid pH and NO_2, both NO carbaryl and N-methyl-urea gave rise to mutagenesis.

Studies are continuing to determine the utility of Drosophila for testing pre-carcinogens. A number of agents which have been shown to be negative or troublesome in the Ames test were mutagenic for Drosophila. Most mutagenic tests employ relatively high levels of mutagens over short periods of time and there remains a dearth of information concerning the adverse effects of environmental agents at low levels for longer periods of time. Preliminary studies to shed more information on this aspect indicate that a relationship between exposure and mutation yield is not easily detectable at low mutagen levels.

In another study which emphasizes an assay for chromosomal breaks as evidenced by the formation of duplication or deletions, Drosophila is being exposed to radiation, EMS and formaldehyde. Since the maroon-like (ma-l) system utilizes an extraordinarily sensitive nutritional selection procedure, it can be used to screen millions of treated chromosomes in a relatively short time. The ma-l may be used to screen suspected environmental mutagens for their ability to damage chromosomes in an eukaryote system.

The identification of genes in Drosophila concerned with mutagenic activity, determination by genetic means of the role of the genes in mutation process and determination of the role of the genes in DNA repair capabilities are also being carried out. The results of these studies should provide basic insights into the genetics and biochemistry of DNA repair and mutagenesis in animal cells. These studies will also serve to characterize Drosophila strains for use in screening environmental agents.

It has been shown that Chinese hamster ovary (CHO) cells are abnormally sensitive to killing by various mutagenic and carcinogenic agents. It is expected that using this system, some understanding of the mechanisms of chromosome repair in mammalian cells and the relationship these processes may have to mutagenesis and carcinogenesis will be obtained. The ultraviolet light sensitive cell (UV-1) was examined for its sensitivity to killing by ethyl methane sulfanate (EMS), x-irradiation, N-methyl-N-nitro-N-nitrosoquanidine (MNNG) and 4-nitroquinoline-1-oxide (4NQO). It was found that the UV-1 cells were more sensitive than the parental cell to killing by EMS and MNNG, but both cell types were equally resistant to x-irradiation and 4NQO. The results indicate that UV-1 cells were more sensitive than the parental cell to killing by EMS and MNNG, but both cell types were equally resistant to x-irradiation and 4NQO. The results indicate that the biochemical defect in the UV-1 cell has significantly altered the cell's ability to cope with the lethal effects of some agents but not to others. If this biochemical defect is in the chromosomal repair system, the results suggest that there may be multiple repair systems which have varying abilities to repair damage induced by different agents. It would appear that different chromosome repair processes have the capacity to repair certain types of damage but not others. The ability of the UV-1 cell to induce single strand breaks in DNA after UV irradiation was also examined. This provides a measure to the cell's capacity to recognize thymine dimers which normally is the first step in excision repair. From these studies, it would appear that the defect in the UV-1 cell is not in the incision step of DNA repair but in the excision mechanism. This is in contrast to most EP cells in which the defect does seem to be in the incision step after irradiation. These studies have significance with respect to individuals with the disease Xeroderma pigmentosum, nearly 100 percent of whom develop skin cancer. Cells from these patients have a reduced ability to repair DNA damage. This correlation suggests that in normal individuals, the rate of DNA repair, compared with other cellular processes could be an important factor in the initial steps of carcinogenesis. Some understanding of the cellular repair process could be important in evaluating the carcinogenic hazard of various chemical agents.

In another study, using mammalian cells in vitro, one of the most significant findings was that biological stains such as, gentian violet and crystal violet, cause chromosome damage. These findings are significant since gentian violet has been used as a topical antiseptic in humans, and also is being mixed in chicken and cow feed to control fungal growth. In several cell systems, different samples of both gentian violet and crystal violet were found to be effective clastogens. Interestingly, when the S-9 fraction of rat liver extract was used in the Ames test, S-9 apparently converted gentian violet into a compound which exerted no mutagenic effect. The S-9 fraction has been shown with a variety of agents to either enhance the mutagenic effect, reduce the mutagenic effect, or cause no effect. Of interest, also, has been the finding that when ethyl alcohol is metabolically converted by the S-9 fraction, chromosome breakage is found in the in vitro test. Using gentian violet as a mutagenic test agent, lymphocyte cells from normal individuals and individuals with a variety of hereditary diseases, such as Xeroderma pigmentosum, ataxia, Fanconi's and Bloom's syndrome, are cultured in vitro for three to four days and exposed to gentian violet. The preliminary results have been encouraging. Normal persons showed an average chromosome break frequency of approximately 1.4 per cell. Persons suspected of genetic instability exhibited at least twice, and in many cases, three times as many breaks per cell.

An interesting approach to the development of a reliable assay and monitor for mutagens and carcinogens in the human environment is the use of the germinal cells of a higher eukaryote Zea mays. Various strains of the maize plants are being tested using different dosages of a number of mutagens. Preliminary results indicate that inbred early-early synthetic is sensitive to chemical mutagens and that acute treatment of early germinating kernels will lead to a response that is measurable in the pollen grains of the mature sporophyte. The resulting data from the mutagen tests should establish the sensitivity of the maize to known mutagens administered to acute or chronic regimens. The resulting genetic crosses are expected to produce lines that will be genetically constructed to serve as monitors for the mutagens.

At the present time, a great deal of effort is being expended in the development and study of tissue culture transformation systems which respond to organic carcinogens. However, few studies are being carried out on inorganic materials. These, also, are considered to be an important problem in industry and the environment. Present studies have confirmed that Ni_3S_2 induces neoplastic transformation and that other nickel compounds which are carcinogenic in experimental animals also induce transformation. The significance of these studies, of course, lies in the development of a simple rapid test for carcinogenic potency of nickel and, perhaps, other metal compounds which would enable one to determine which compounds can, and which ones cannot, potentially cause cancer in humans.

A test for detecting environmental carcinogens utilizing the frog as an experimental model, is under development. The objective is to devise an in vivo test system in a vertebrate which can provide rapid results. The model compound, triethylenemelamine (TEM) is being used to induce structural chromosomal abnormalities using a dominant lethal protocol. Morphological abnormalities in the progeny of males treated with the compound are detected. The system appears to show a greater degree of sensitivity than comparable mammalian systems with the model compound selected. Embryos are being scored for defects and appear to relate to the dose of TEM. One function, swimming, could also be utilized in the scoring system which relates to the dose of TEM to the parent. Cytogenetic observations also showed dose-related effects.

AIR POLLUTANTS

Alveolar macrophages and sensitized lung fibroblasts were exposed to ozone to determine the extent of injury imposed upon these cells. The most significant finding during the past year was that the growth of lung fibroblasts was significantly inhibited by O_3 concentrations as low as 0.05 ppm for a net exposure time of 30 minutes. This affect was prevented by an overlay of 1.5 millimeters of balanced salt solution, suggesting that the products of O_3 with the aqueous medium were not toxic under the conditions used.

The alveolar macrophages which have powerful intercellular digestive systems and metabolic activities are the primary defenders of mammalian lungs against airborne infection. Biochemical studies on the effect of iron oxide aerosols on activation of the lysosomal system of alveolar macrophages suggest that breathing the particles stimulates phagocytosis as well as marshalling of macrophage reserves to increase the overall digestive capacity of the macrophage population present.

The combination of gaseous and particulate pollutants containing oxides of sulfur and oxides of nitrogen with levels three to five times the federal standards have been investigated to determine the effects on pulmonary defense systems. It has been determined that the biological systems which maintain pulmonary sterility, e.g., mucociliary, transport and alveolar macrophages were not impaired in their ability to inactivate staphylococcal or streptococcal bacteria in rodents.

A technique to better measure material balance in inhalation deposition studies has been developed. Reproducible results have been obtained from a new device to measure sizes of exhaled aerosol particles. This device will assist in measurement of exhaled particulates in studies of deposition patterns in human and animal airways.

A study concerned with the factors influencing deposition of inhaled particles has shown that the increases in aerosol deposition which occurs during exercise, are greater than those which can be attributed to increase in ventilation alone. Not only do more particles enter the respiratory tract with exercise, but a greater fraction fail to exit.

When sacrifice and analysis was delayed 24 hours in some groups of animals to allow time for upper respiratory tract clearance, aerosol retention was not dramatically lower than in those that were sacrificed immediately. These findings showed that the submicronic aerosols used were deposited deep in the lungs because little was cleared by the mucociliary transport system. This research has also developed techniques for quantitative measurement of deposition of a radio active aerosol by autoradiography. In this same program, an attempt has been made to determine the effect of smoking on clearance of magnetic dust from human lungs. The long-term clearance of dust from human lungs was measured in three heavy smokers of cigarettes and nine non-smoking subjects, using trace amounts of magnetic dust Fe^{304}. Clearance curves describing the disappearance of the dust showed that after 11 months, about 50 percent of the dust first deposited, remained in the smokers, while only 10 percent remained in the non-smokers; the smokers therefore retained five times more dust. This impaired long-term clearance in smokers seen for the first time, may contribute to the higher incidence of some lung diseases in smokers. It is suggested that smokers might also have a higher retention of toxic occupational and urban dust.

A major study continues on the effects of SO_2 and respirable particulates on health as determined in a six-cities-research project. Preliminary results indicate that an examination of lung function and disease rates shows a marked effect of cigarette smoking. At the present time, the results also show significant differences for the various parameters which are being studied between the various cities. Once a model has been established that takes into consideration all the relevant parameters, more meaningful comparisons can be made between the cities.

An epidemiological study has just been concluded, which was designed to study acute health effects of air pollutants, by the methods of multiple regression of health indices on pollutant concentrations in New York City. The study demonstrated that there are a number of serious methodological problems facing researchers in the field of acute health effects of air pollution. These include: the interaction between pollutants both in the atmosphere and after they have entered the human body; synergism between, or potentiation of the biological activity of environmental factors; the accuracy and validity of air pollution measurements, in particular, at current levels of air pollution; and the question of indoor exposure to air pollutants in view of recent demonstrations of 100 to 1,000-fold greater exposure relative to outdoors. This has been shown to be particularly important in homes using gas for fuels or in homes in which there are individuals who smoke. In addition to the identification of problems involved in the methodology, these studies have demonstrated the inadequacy of using the New York City aerometric network to represent daily exposure of the population, and the identification of the so-called "asthma-alley" in an area of the city in which higher rates of asthmatic attacks occur.

A study is underway to determine whether the natural history of chronic obstructive respiratory disease (CORD) and functional respiratory impairment correlates with long-term exposure to different types of air pollutants in four communities. An effort will be made to measure the sensitivity and specificity of selected tests of lung function for the identification of early respiratory impairment and CORD. During the past year, analysis and comparison of results of lung function tests in three study areas have been completed. The results suggest that residence in areas exposed to photo-chemical/oxidant or SO_2 hydrocarbons/particulate pollutants is associated with impaired lung function, probably related to the large airways. In the two polluted areas, the spirometric indices and the plethysmographic indices were worse. Again, with respect to large airway performance tests, there seems to be an association with residence in an area exposed to air pollutants. Confirmation of the results of the cross sectional studies should make it possible to identify pollutants to which long term exposure results in impairment in lung function.

The behavioral effects of exposure to levels near the TLV of carbon monoxide have been measured. The objective of this research is to determine minimum dose and exposure times which cause effects for the pollutants carbon monoxide, nitrogen dioxide and sulfur dioxide. Upon such levels of exposure, baboons showed a slowing of response time in a learned behavior. This also demonstrates the sensitivity of the test system being used.

The consequences of prenatal carbon monoxide exposure on subsequent behavior and performance, neurochemistry, and cellular structure is also being studied. Differences in learning ability of exposed rats have been observed, as has hypertrophy of neonatal heart muscle in exposed animals.

The effects of mild, sublethal exposures to pulmonary oxidants, such as oxygen, ozone, and paraquat are under investigation. Sublethal doses of the three materials yielded similar lesions, but with distinctly different temporal patterns. The role of pulmonary P-450 in these patterns is under study.

An attempt is being made to determine which oxidations occur in tissues exposed to environmental oxidants, particularly in smog and polluted air, breathed into the lungs or continuously in contact with the skin and eyes, etc. Singlet molecular oxygen among other agents, is known to be an injurious oxidant in such polluted air. However, the extent of the activities of this agent is not fully appreciated. For example, it is of interest to be able to differentiate between singlet oxygen products or cholesterol-free radical auto-oxidation products and products of other oxidation processes. High performance chromatic separations are being developed for the analysis of complex mixtures of tissue for cholesterol auto-oxidation products.

There is experimental evidence to suggest that singlet molecular oxygen can form in the lower atmosphere and persist long enough to reach the lungs where it might initiate an inflammatory reaction. In addition, singlet molecular oxygen can react with atmospheric olefins to form toxic hydroperoxides. These substances also may initiate a pathologic response upon reaching certain sites in the lungs. Techniques are being developed for producing and handling singlet molecular oxygen at atmospheric pressure.

Even though it is believed that many industrial chemicals cause pulmonary hypersensitivity in workers, there is no reliable test for predicting which industrial chemicals might be allergenic for man. A study is underway to develop a guinea pig animal model for predicting the sensitizing abilities of industrial chemicals. Hapten-specific respiratory hypersensitivity in guinea pigs has been produced using three aromatic chemicals, picryl, p-azobenzene arsenate and p-tolyisocyanate. A small aliphatic chemical hexyl isocyanate has also been used to sensitize guinea pigs as a corollary to the aliphatic reagent, hexamethylene diiscyanata which is reported to cause hypersensitivity in industrial workers. The animals have developed pulmonary hypersensitivy directed toward hexamethylene diisocyanate. It is apparent from these studies that the guinea pig model provides a method whereby the sensitizing potency of both aliphatic and aromatic industrial chemicals can be compared when protein is also being investigated, using such proteins as ovalbumin, ribonuclease and guinea pig serum albumin. In addition, an antigen has been prepared and shown to be effective in detecting antibodies to toluene diisocyanate (TDI) in workers. Sera has been collected from a large number of workers and will be tested to determine the relationship between the TDI antibodies and the degree of pulmonary sensitivity to TDI.

A series of experiments in which Sprague Dawley female rats were exposed for three hours to a constant halothane concentration revealed large amounts of volatile halothane metabolites in the liver of these animals and less in the lungs and kidneys. The volatile halothane metabolites were identified as: CF_3CH_2CHCL. It was found that the larger the exposure concentrations, the larger the relative concentration of halothane and the smaller the relative concentration of metabolites in the tissues. Metabolite concentrations in the lungs and kidneys were approximately 1/10 of the concentrations in the liver. Only traces of metabolites were found in blood, brain, muscle and fat of rats exposed to smaller concentrations. Metabolite concentrations in these tissues were not detectable. The clearance of the metabolites from the tissues by blood was found to be slow and therefore, significant amounts were found in tissues in which they were formed.

METALS

The effects of selenium deficiency with regard to the role of selenium in heme metabolism, is under investigation. In rats, a survey of hepatic selenoproteins was undertaken to determine which ones influence heme metabolism, and to examine the role of nonselenium-dependent glutathione peroxidase. Several different proteins from selenium-deficient rats have been isolated and are being compared with similar proteins from control rats. The influence of these proteins on the destructive process of lipid peroxidation is currently under study.

In another study of the mechanism of selenium toxicity, the metabolism of selenium in plants which tolerate this material is being compared with other organisms which are sensitive to selenium. The mechanisms are being investigated on a molecular level to determine the ability of the organisms to use selenoaminoacids as substrates for the formation of aminoacyl-tRNA and the formation of proteins. Initial evidence shows that sensitive plants fail to discriminate against selenoaminoacids, thus leading to the synthesis of aberrant proteins.

Cadmium is used in electroplating, in the production of alloys and pesticides, and in pigment formulation. Motor vehicle exhausts and industrial effluents contribute to the atmospheric burden of cadmium. Cadmium is a by-product of zinc and lead production, and due to the increase in its use, the potential for environmental exposure continues to grow, particularly in the heavy industrial areas. A study is underway to elucidate the neurotoxic effects of low levels of cadmium exposure during the pregestational, neonatal and adult periods on selected neurophysiological and behavioral parameters. The presence of cadmium in the drinking water during gestation only, is as effective in promoting behavioral changes in pups, as exposures which include pregestational as well as exposure during gestation. Exposure during pregestation had no detectable effects. From this, it appears that the period of gestation is the critical time to cause increases in locomotor activity in pups. Aggression seemed to increase, and sensory and motor nerve conduction velocities in the sciatic nerve failed to demonstrate any deleterious effects in those animals previously exposed to cadmium. The latency and amplitude of responses recorded from the cerebral cortex evoked by sciatic nerve stimulation were also not altered by cadmium exposures. However, another index of central nervous function, the recovery function of the inner hemispheric response, was depressed in cadmium-exposed rats.

METALLOTHIONEIN

The effect of gestational exposure to cadmium has been evaluated behaviorally by means of a simultaneous visual discrimination task, with reversal. Adult rats exposed gestationally to 17.2 ppm cadmium were found to be deficient in acquisition of the reversal phase. Therefore, the effect appeared to be quite subtle, but also persistent. The mechanisms of action of such an effect, although not known, must be due to some early indirect action of cadmium, since exposure was terminated at birth.

A project to investigate the absorption, excretion, and sequestration of cadmium is yielding some useful information. Doses of radiolabeled cadmium chloride administered by intubation, indicated that only approximately 1 percent of the material was absorbed. After initial deposition of the absorbed cadmium, the isotope was depleted from all tissues except the kidney. Although high concentrations initially were found in liver, pancreas, spleen, and testes, it is hypothesized that the retention in these tissues is related to the presence of metallothionein. To determine the presence of metallothionein, antibodies to rat hepatic Cd (metallothionein) were produced in rabbits. This method shall be employed to measure metabolism in biological tissues in its role as a barrier against the transplacental passage of cadmium.

The toxicological significance of cadmium-metallothionein (Cd-Mt) is being determined by experiments to learn how the compound is handled by different tissues. In the kidney, it was found that renal filtration was followed by re-absorption of Cd-Mt and therefore, this mechanism is insignificant in cadmium excretion. Tubular secretion of Cd-Mt was also found to be insignificant or extremely slow; therefore, it appears, at this point, that complexation of cadmium with metallothionein is not a mechanism by which the organism enhances the urinary excretion of the toxic metal.

It has been determined that livers from newborn rats contained concentrations of metallothionein approximately 20 times that of older rats. Also, a suspected association between liver glutathione and metallothionein was a spacious relationship. Treatments causing immediate depletion of reduced glutathione also increased hepatic metallothionein. However, the effect was delayed and induction of hepatic and renal metallothionein caused no concomitant reduction in glutathione.

A cell-free protein synthesizing system derived from wheat germ extracts which is capable of synthesizing metallothionein from liver polysomal mRNA has been developed. In this system, metallothionein mRNA can be induced by injections of either zinc or cadmium. This system has shown that metallothionein mRNA is found on free polysomes rather than on membrane-bound polysomes, suggesting that metallothionein is an intercellular protein. The model system also responds to zinc induction, indicating that group IIB metals induce metallothionein mRNA. This should provide an excellent model for studying the toxicity of these metals.

Another study involving metallothionein involves the establishment of an _in vitro_ cadmium-binding protein (CdBp) synthesizing system. The material can be purified by chromotography and is identical to standard CdBps synthesized _in vivo_. It has been determined that although the cadmium protein complex is degraded over time, the released cadmium is rebound to more newly synthesized metallothionein chains. This may explain why, in spite of this degradation, cadmium is retained in target tissues for extended periods. Cadmium retention was also found to be enhanced by feeding low-calcium diets because the low-calcium stimulus activated release of parathyroid hormone which, in turn, enhanced calcium and cadmium absorption.

The chemistry, biochemistry and physiological chemistry of metallothionein are being studied to clarify their role in essential metal metabolism and heavy metal toxicity. Using Ehrlich tumor cells injected with cadmium chloride, a 10,000 molecular weight cadmium-binding protein has been identified. Interestingly, about 50 percent of the cellular Cd is found in the particulate fraction of the cell, similar to the findings using rat liver. These studies have also shown differences in transport properties of zinc and cadmium which parallels differences in the turnover of these metals in organs. The difference being that while both zinc and cadmium are taken up into cells in fresh metal media, zinc has an efficient efflux mechanism, whereby cadmium remains bound in the cell. The tumor cells exposed to cadmium will contribute to our understanding of how cadmium interacts with cells and the role metallothionein plays in these reactions.

In another study, time courses of induction of metallothionein and chelatin in rat liver under in vivo conditions were carried out for cadmium, zinc and copper induction. These studies should provide a better understanding of the processes involved in heavy metal metabolism, metallothionein and its role in the metabolism of metabolically essential and toxic heavy metals. A single intraperitoneal injection of mercuric chloride led to the synthesis of zinc thioneine in rat liver with a peak of induction at 18 hours, similar to that which was seen with zinc induction. No mercury was found to be attached to the purified metallothionein after induction by mercury. Actinomycin administered at the peak of induction led to a two-fold increase in the level of zinc thioneine, 12 and 23 hours afterward, relative to controls receiving no inhibitor. The behavior of the induction of zinc thioneine in rat liver by mercury is similar to that seen with induction by zinc and copper but is different from that seen with induction by cadmium. The production of a rabbit antibody, monospecific for rat zinc has successfully been elicited. Full development of a radioimmunoassay for rat liver thioneine is currently underway.

The ubiquitousness of lead in the environment and its associated problems continues to be of interest to environmental health scientists. The major use of lead to which human populations are exposed include: storage batteries, fuel additives, and pigment manufacture. Chronic or sub-acute lead exposure leads to neurological, renal, and hematological manifestations of disease.

An animal model system has been developed using radiolead which permits studies of the absorption, excretion, and body distribution of lead at intervals after the administration of either oral or parenteral doses of the material. The absorption of lead has been found to occur almost solely from the small intestine and seems to be maximal in the duodenum, whereas bile enhances the transport of lead across the intestinal mucosal epithelial cells. In the rat, maximum uptake occurs within one-half hour following the administration of a test dose. The system appears to be saturable, since increasing amounts of lead in the test dose were associated with decreases in the percentage of radiolead which was absorbed. The total body burden of lead did not seem to significantly affect lead absorption. Although the GI tract serves as an important route for the excretion of lead, there does not appear to be a feedback mechanism to limit absorption of lead, as is seen with other materials. Also, young animals were observed to have increased absorption. Excretion kinetics and the role of dietary calcium is also under investigation in this study.

Studies have clarified the importance of low-level exposure to lead. Impairments of mitochondrial function with accumulation of substrates of mitochondrial reactions in the circulating erythrocytes have been found at lead levels observed in normal urban dwellers, both children and adults. The techniques used for this observation may possibly be adapted as a diagnostic technique for lead poison.

It appears that the inhibition of red cell pyrimidine^5 nucleotidase may be established as another zero-threshold effect of environmental lead, whereas the accumulation of pyrimidine nucleotides in the red cells may have a higher threshold, possibly one that parallels other health effects. These effects are being studied clinically. The biochemical significance of the inhibitory effect of lead on pyrimidine^5 nucleotidase and its molecular relevance to the impaired degradation of RNA associated with basophilic stippling in altered red cell metabolism, is under study.

The potential for using computer-assisted radiographs to determine from bone densities whether or not children have been exposed to lead, is being developed. This method would allow for screening for chronic exposure to lead, as opposed to blood analyses which will indicate acute exposures.

Pregnancy studies have shown that fetuses born of lead-fed rats have a reduced body zinc content. Also, lead interferes with the metabolism of zinc, copper and iron, and produces congenital abnormalities. It will now be determined if zinc supplementation of the rat dams during pregnancy prevents these effects. If so, a rationale for possible prevention will exist.

About four out of ten adults who become lead poisoned develop gouty arthritis (saturnine gout). Following on the observation that guanine aminohydrolase (guanase) is exquisitely sensitive to lead, it has been hypothesized that the precipitation of guanine rather than uric acid could be the cause of this gout. Due to the deficits of guanase in the domestic pig, this animal was utilized as an experimental model for inducing saturnine gout. Lead acetate administered by injection to this animal yielded a five-fold increase in guanine excretion over a period of 48 hours. After sacrifice, the epiphyseal plate of the femoral head revealed concentrations of needle-shaped crystals which contained substantial amounts of guanine. Control animals did not show any crystalline concretions nor any guanine in extracts of the bony tissues.

In one such study, in anticipation of the expected increase in the lead levels in the environment, the effects of lead intake by children and the adverse effects of lead accumulation on neuropsychological development is being pursued. Utilized in this study are longitudinal, neuropsychological, behavioral and biomedical data collected prospectively by the collaborative perinatal project and assessments of current neuropsychological functioning.

Analysis of lead content in the primary and secondary dentin of the subjects' exfoliated decidious teeth are providing an index of lead accumulation over time. By integrating these two sets of data, attempts are being made to determine whether lead exposure at various ages, singly or in concert with other developmental events, produces deficits in neuropsychological functioning in children. These studies utilize atomic absorption spectrophotometry for lead analysis of the teeth.

A project is being carried out to define various factors which determine the movement of lead into and out of bone, including hormones, ions, and vitamins. Metal-hormone-vitamin-nucleotide-collagen interactions are being studied in bone organ culture and in parathyroidectomized rats to test the relevance of the cell culture findings. In cell culture, EDTA produces solubilization of bone material and matrix qualitatively similar to the action of parathyroid hormones. Brief exposures to parathyroid hormone or EDTA and transfer to medium without either agent, brought about a release of lead. Lysomal mechanisms were also determined to play an important role in the release of previously incorporated lead from bone into the medium. Agents, such as glucocorticoids and vitamin A had marked effects on lead transport. In parathyroidectomized rats infused with EDTA or a parathyroid hormone, it was determined that parathyroid hormone produced an increase in brain-lead content. On the other hand, calcitonin inhibited EDTA's action by checking removal of lead from bone. Clinically, preliminary evidence was developed that children with mild to moderate undue absorption of lead have low circulating levels of dihydroxy vitamin D compared to normal controls. Thus, the enhancement of lead toxicity in children with marginal nutrition is suggested.

The effects of chelating agents, EDTA, ascorbic acid, and their combinations in removing lead from poisoned rats have been compared. The combination therapy was found to be more effective than single therapies with either agent. EDTA seems to remove more lead from the liver, whereas ascorbic acid may be more effective in removing lead from the brain. However, EDTA is also more effective in reducing kidney and possibly bone lead than ascorbic acid alone. Combined therapy does not appear to increase the potentially-toxic effects of EDTA. However, additional studies are needed to determine the effects of accompanying losses of essential metals, such as zinc.

Using monolayer cultures of glia and neuroblastoma cells as an experimental model, the molecular mechanisms of heavy metal-induced damage to nervous tissue is being investigated. Low concentrations of methylmercuric chloride caused extensive modification of gene expression as evidenced by alterations in the amounts and net phosphorylation profiles of specific proteins. The findings suggest that molecular lesions can be observed in mercury-treated cells against the cytotoxic effects of methylmercury chloride, whereas vitamin C seems to potentiate the affect on neuroblastoma cell tissues. It was also observed that mercury acted synergistically with lead to inhibit guanase and is readily reversible by EDTA or BAL indicating that guanine gout due to lead toxication could be reversed by standard inhalation therapy.

The major reservoir of lead in humans is the skeleton, yet relatively little is known concerning the molecular actions of this toxic metal in bone and the mechanism of removal of this metal by various chelators. Since exhanges between body compartments of lead are likely to be rapid in growing children due to the rapid rate of bone remodeling, information on factors which effect mobilization of lead from bone are important to know. Studies to understand metal and hormone-enzyme effects on this mobilization, as well as nutritional effects (especially vitamin D) suggest that all of these factors may be important in reducing the toxic effects of lead in children.

Comparison of the findings of the tissue-lead analyses with the neurobehavioral studies revealed that subtle abnormalities during development and adulthood resulted from relatively low-level lead exposure limited to the early postnatal period. The results have significance for future work and for human health problems. Exposure conditions suitable for further investigation, particularly of hippocampal function, have been identified. The 0.02 percent level of lead exposure does not impair body growth but does result in reliable neurological abnormalities. The 0.2 percent exposure level produces greater neurological disturbances and produces only slight reduction in growth. Additionally, several screening tests were found to be sensitive to lead's neurotoxicity and these measures will be useful in the initial assessment of modification of lead's neurotoxicity via manipulations of dietary zinc levels. Implications of this work for human health are that subtle disturbances in neuromuscular ontogeny and CNS convulsability are sequelae of relatively brief, low-level lead exposure during neonatal development. These effects were observed in animals that were overtly free of poisoning symptoms, exhibited normal growth, and had maximum blood lead concentrations below 50 μg percent. It has been estimated that there is a large pediatric population undergoing low-level lead exposure. These children remain overtly asymptomatic and have blood levels below 50 μg percent. Effects on the developing nervous system comparable to those reported here in the rat, may occur in these children.

Acute and persistent neurobehavioral effects of sub-clinical lead exposure during the main periods of neurobehavioral development in the monkey is being determined. Correlations are also being made with the various biochemical and clinopathological assessments of lead damage in the monkeys. Controversy exists over the role of milk as a prophylactic for lead poisoning, and during the past year, studies have indicated that feeding of high milk diet may be exacerbating the toxic effects associated with a given level of lead. Additional studies suggest that animals previously exposed to lead in the diet and subsequently free of lead exposure for a three-day period have a permanent deficit which has worsened with neurobehavioral maturation. It would appear that the behavioral nature of the deficit hinted at hippocampal damage. This area of the rat brain is known to concentrate lead seven-fold over that of other brain regions. The significance of this study resides in the fact that numbers of people in this country and especially children, carry blood lead levels in the range being studied. The health hazard of these sub-clinical lead levels are not well understood. It is known that severe acute lead toxicity produces neuropathies, visual system damage, and severe intellectual impairment. Studies are underway to determine the chemistry, biochemistry, and physiological chemistry of metallothionein in order to clarify its role in metal metabolism and heavy metabolism and heavy metal toxicity.

Chemical studies have demonstrated that the 10,000 molecular weight fraction of metallothionein from rat livers of animals grown under chronic exposure to cadmium has additional binding capacity. This is perhaps the first example where this fraction has been shown to be unsaturated in metal content. These investigators feel that the Ehrlich cell model offers a controlled system for the examination of cadmium and zinc-cadmium biochemistry. They have reported the first example of a 10,000 molecular weight zinc-binding protein which exists in the absence of external zinc stress. One striking finding has been that a short 20-minute incubation of cells with cadmium in vitro is sufficient to bind cadmium to a 10,000 molecular weight zinc-binding protein which exists in the absence of external zinc stress. One striking finding has been that a short 20-minute incubation of cells with cadmium in vitro is sufficient to bind cadmium to a 10,000 molecular weight protein. In this system, cadmium is distributed throughout the cell with only 50 percent of it in the soluble 10,000 molecular weight protein.

The effect of ingestion of methylmercury by pregnant women on offspring is under investigation. It has been demonstrated clinically that such ingestions cause serious CNS damage to unborn babies leading to various cerebral palsy syndromes accompanied by mental retardation. The mechanism of these effects is being studied by the utilization of tissue cultures to observe the development of the brain under conditions of exposure. Several preliminary effects of such exposures are as follows: (1) cessation of neuronal migration and alteration in proliferative activity, and morphology of astrocytes; (2) cessation of movement of filopodia on tip of neurites of small immature neurons; (3) disappearance of neurotubules with bundles of neurofilaments; and (4) cessation of proliferative activity of astrocytes. These all support the working hypothesis that a major effect of prenatal methylmercury intoxication in developing human brain is related to abnormal neuronal migration and deranged cortical organization.

New therapies for acute and chronic mercury poisoning using chelating agents are being developed. Water soluble chelating agents with three categories of donor groups have been synthesized: dimercaptols, dithiocarbamates, and a substituted phosphine. These compounds are being compared in their ability to alter lethal doses of mercury chloride. Comparison is being made with the best of the current chelating therapies for acute mercury poisoning. Several promising compounds have been discovered and it is important that those with sulfur donor atoms also possess a high water solubility which generally will indicate a lower inherent toxicity and a higher selectivity for mercury ions that alternate donor systems.

PHYSICAL FACTORS

Noise

Noise studies are hampered by the large degree of variability encountered in individuals following exposure to noise. The threshold shift from one individual to another may be enormously different and as yet, unaccountable on the basis of the fine parameters. Explanation for the significant variability is being sought in order to be able to explore more significantly the effects of interacting continuous and impulse noise, noise-drug interactions, noise-vibration and methods to identify, analyze and understand the pathology produced by ototraumatic agents.

Preliminary studies concerning the noise vibration interactions, of great potential significance in terms of occupational hazards to man have shown that using the chinchilla as a model, 1-G vibration of the head at 30 Hc during exposure to 50 impulses at 158 Db (40 microsecond A-duration) increased the damage to the cochleas and the size of the permanent threshold shifts compared to the effects of the noise or the vibration. From these preliminary studies, it appears that an increased hearing loss occurs after the addition of vibration; however, this conclusion is felt to be premature in view of the variability encountered in the subject animals.

Attention is being focused upon the qualitative and quantitative relations between characteristics of noise and noise-induced temporary hearing loss, and to identify and understand the audiometric correlates of noise-induced hearing loss, by exposing human subjects to a wide band noise for 24 hours intermittently. Regardless of the on-time for duty cycles, the threshold shifts (TTS) after 24 hours of exposure were nearly identical with those measured after eight hours, although the TTSs increased for about 8 to 12 hours. The magnitude of the TTS was related to on-time and duty cycle in a complicated manner, i.e., for on times greater than 4,000 seconds (66 minutes) the magnitude of TTS approached or equaled the TTS reproduced by a steady state exposure. For on-times, less than about 1,000 seconds but greater than 10 seconds. TTS is directly related to duty cycle. For on-times of one second or less, and duty cycle greater than 50 percent, TTS appears to approach the TTS produced by a steady-state exposure.

Microwave Irradiation

Exposure of the general population to government, industrial and commercial sources of chronic low-levels of microwave irradiation has been increasing significantly over the past decade. The effects of chronic, low-level microwave irradiation at 915 and 2450 MHz upon pregnant females have been analyzed to determine whether the embryo can be utilized as a sensitive indicator of the non-thermal effects of this form of radiation. The present data indicate that there appears to be no observable adverse effects on an organ or behavioral level to rats exposed *in utero* for 8 hours/day throughout pregnancy at a whole-body, non-thermal irradiation level of 10 mW/cm at a frequency of 915 MHz, or 20 mW/cm at frequency of 2450 MHz. There are, however, several questions which are still unresolved. Are the minor changes which were observed significant? Are there structural correlations which could be made? Can rats perceive microwave radiation at these frequencies? What is the nature of the thermoregulatory mechanisms in the rat? Is the blood brain barrier alerted by exposure to microwaves?

Studies are underway to characterize the responses of auditory single units to post-microwave irradiation. This type of interaction can be observed at low average incident power levels and, in fact, an effect can be observed in response to a single brief pulse of MWR energy. Preliminary findings with respect to exposure to microwave irradiation indicate that a mechanical wave front is launched into the head by the MWR pulse, but the degree to which that is exclusively a high frequency (20 to 40 Khz) mechanical wave requires more detailed study. These data seem to indicate that a significant low frequency component must also be considered in the interaction.

High Voltage Transmission Lines

A project is underway to study and quantify the biological effects of 60 hertz electric fields to provide a basis to assess the degree of risk of exposure to such fields emanating from high voltage transmission lines and from ordinary household electrical devices. The results show that: (1) definite field-induced changes in body weight and mortality were observed in three generations of mice continuously exposed to 3.5 kv/m at 60 hertz; (2) bone fractures were observed to have retarded healing when animals were exposed to 5 kv/m for fourteen days; (3) sunflowers exposed to 5 kv/m for seven days exhibited increased mortality and increases in average plant height; and (4) using mathematical models of a human being, penetration of electromagnetic energy at 60 hertz is greater than predicted by less sophisticated means.

Photosensitivity

Studies on environmental photosensitizers are directed toward a complete evaluation of designated agents. Because all aspects -- location in tissue, photochemistry in tissue and experimental animal studies -- are coordinated, the opportunity for increased understanding is enhanced. Of direct significance to the area of research of light sensitivity in man is evidence for a new mechanism for photoxicity, by which photoproducts from the photosentizer (chlopromazine and protriptyline), non-oxidatively damage cell membranes. Also of significance to health problems is an evaluation of musk ambrette as a contact photo-allergic but not phototoxic agent. Chlorpromazine and protriptyline, two photosensitizers, used as psychotherapeutic drugs, have been tested in a red blood cell photohemolosis system. Both compounds photosensitized the lysis of red cells in the presence and absence of oxygen. In contrast, all other membrane-damaging photosensitizers require oxygen to photohemolyse red cells. These results indicate that chlorpromazine and protriptyline represent a new mechanistic class of phototoxic compounds. It was further demonstrated that the lysis was due to photoproducts of chlorpromazine and protriptyline which can be produced in the absence of red cells. Addition of these photoproduct mixtures to red cells suspensions lysed the cells without causing chemical changes to the unsaturated fatty acids of the membrane phosphlipids. The lytic photoproducts are as potent as, or more potent than, detergents, such as sodium dodecylsulfate and dodecyldimethylamine oxide.

Compounds which are constituents of coal tar preparations used in the photochemotherapy of psoriasis (anthracene, acridine, phenanthracene, chrysene, carbazole and fluoranthene) were tested at concentrations between 5 x 10^{-3}M and 5 x 10^{-6}M in ethanol. Only anthracene and pyrene were concluded to be statistically significant phototoxic agents when tested with lights in the UVA (320-400 nm) range.

NATURAL TOXINS

A chemical approach to understanding some aspects of the mechanism of action of substituted furans (pulmonary toxins) is in process. Within this class of compounds is a 4-ipomeanol and its derivatives (compounds isolated from sweet potatoes). Subjecting a number of these compounds to the Ames bacterial system indicated no mutagenic activity. The carcinogenicity of 4-ipomeanol was also tested in a rainbow trout embryo system. Results were also negative.

It has been determined that toxins from jelly fish (*Stomolophus meleagris*) interfere with ion transport across membranes and with mitochondria function. *In vivo* effects on ATPase activity on calcium uptake were measured and found to be significant. The specific compound responsible for the defects is currently being determined and isolated.

Studies on the mechanism of action and of the toxification of rubratoxin B are in progress. It has been determined that rubratoxin B produced significant decreases in hepatic levels of cyclic AMP in mice. However, the effect was not seen until 18 hours after treatment, and thus, is not associated with the rubratoxin B induced decrease in liver glycogen. Pretreatment of mice with phenobarbital reduced the toxicity as measured by the LD50 while SKF 525A pretreatment enhanced the toxicity. These studies suggest that the mycotoxin, itself, is the toxic agent, rather than a metabolite.

It has been determined that a material produced by the blue-green alga Gomphosphaeria aponina (called aponin) is cytolytic toward the red tide organism Gymnodinium breve. This material is not being purified in an attempt to chemically characterize the substance for potential use against red tide blooms. It has been learned that chromatographic separation results in a number of fractions but each of these were of reduced specific activity. Therefore, it appears to inhibit the red tide organisms. The mechanisms of action of this inhibition are currently under study.

Isolation and identification of the toxins produced by the red tide dinoflagellate Gymnodinium breve is being attempted. Infrared high-pressure liquid chromatography, nuclear magnetic resonance and mass spectroscopy are being utilized to attempt to identify the components and possible formulas of extracts. Associated physiological studies are being used to determine the activity and mechanisms of the toxins. It has been determined that the toxin interacts with sodium channels by modifying membrane currents following the action potential. Repetitive firing is induced with G. breve toxin and the effect was not reversed by washing. Also, the material inhibited uptake of calcium by sarcoplasmic reticulum vesicles.

The potential danger to human health caused by the cysts of the toxic dinoflagellate Gonyaulax excavata (tamarensis) is being studied. The study is based on the development of an understanding of the process and products of encystment. The approach is to define the fate of both the temporary and the resting cysts of G. excavata in the marine environment, including measurement of viability, and secondly, to measure the fate of the toxic products from these dinoflagellate cysts in the marine environment. The investigations encompass: (1) field measurements whereby viability will be assessed and initial attempts at mapping of hot areas, as well as (2) laboratory studies whereby conditions are controlled so that one environmental parameter at a time, as well as synergistically, will be measured within the natural ranges found within the Gulf of Maine. Where found, cysts behave as fine silt particles within the sedimentary regime; some areas remain relatively cyst-free. These sedimentary factors raise important questions regarding how PSP might be spread by man's activities in the seas; in particular, those emerging from marine dredging. These effects may be manifested in a wide range of marine activities. Those engaged in projects involving artificial seeding of shellfish beds, shellfish culture, and marine dredging operations should be alerted to possible dangers. Dredged-up sediment should be presumed to contain toxic cysts which may be carried far from the source after dumping at sea. Caution should be used when transferring shellfish from one area to another since microscopic cysts can easily be carried with them. It should be emphasized that once introduced into a new area, cysts may directly contaminate shellfish and thus may not only toxify the shellfish extremely rapidly (particularly in late summer) but may establish more permanent local toxic dinoflagellate populations.

An attempt is being made to relate the molecular structure of botulinum toxin to its toxicity. The study is concerned with the components of the toxin molecule and how these parts (naturally or experimentally produced) fit together to produce the well-known high lethality of this toxin. The different antigenic types of botulinum toxin are expected to have basic structural similiarity. Types A and B toxins were subjected to separate, controlled digestion with trypsin and chymotrypsin. The L chains of both toxins were very resistant to trypsin but chymotrypsin degraded the chain to some extent. The important initial change was that of both enzymes acting on the H chains of the two toxins, the H chain was cleaved near the midpoint with the resulting appearance of 50,000 mol. wt. fragment (SCF) plus a 100,000 mol. wt. dichain composed of the original dichain missing SCF. SCF was then cleaved with a -S-S loop. When all of the original dichain had lost its SCF, toxicity had disappeared. The studies may prove that botulinum toxin belongs to the category of bacterial and plant toxins which have different toxic and receptor subunits (e.g., A and B fragments of diptheria toxin). If the H and L chains can be separately purified, it should be possible to produce antisera specific for each subunit. Antibody to one chain could prove more effective than that to the other in treating botulism. The need for better therapeutic antitoxin is emphasized by the large botulism outbreaks in 1977 and 1978, each involving more than 30 cases. The increasing number of infant botulism cases being identified also points to the need for better understanding of the toxic molecule.

In kidney cortex slices from rats fed 2.0 mg. ochratoxin A/kg/day for two days, gluconeogenesis from pyruvate is reduced 26 percent, while renal phosphoenolpyruvate carboxykinase activity is lowered by 55 percent. Gluconeogenesis from 10-20 mM lactate, malate and glutamine is also significantly increased, and hexokinase activity in kidney and liver remains unaffected. These studies have demonstrated that ochratoxin A is a selective in vivo inhibitor of renal phosphoenolpyruvate carboxykinase activity of renal hexokinase. It is likely that this inhibition parallels the fact that ingested ochratoxin A is exclusively localized in the proximal convoluted tubules of kidney where gluconeogenesis is the major metabolic pathway and is rate-controlled by phosphoenolpyruvate carboxykinase activity. These results, as well as preliminary experiments examining the effect of ochratoxin A on total protein synthesis in renal cortex, suggest that the activity of other enzymes localized in proximal tubles may be decreased through inhibition of protein synthesis by ochratoxin A.

BIOMECHANISMS

Cell recovery from exposure to organophosphate insecticides is under investigation by examining their actions in cultured target cells, such as muscle and nerve. The focus of these studies is upon the effects of these chemical agents on the regulation of acetylcholinesterase (AChE) and other molecules at the neuromuscular junction and on the general synthesis and degradation of proteins found in these cells. It has been found that nerve cells respond to poisoning by diisopropylfluorophospate (DFP) and to agents that stimulate the AChE levels in ways similar to muscle cells, demonstrating that contractility is not the sole mechanism by which muscle cells regulate AChE activity. Circumstantial evidence has now been obtained indicating that chemicals which alter ion movement in muscle cells regulate AChE activity. Through a number of follow-up studies, it was proposed that there is a "regulatory receptor" on the surface of cultured muscle, and probably also nerve cells, which acts to control the levels of macromolecules destined to function at the myoneural junction. The pharmacological properties of such a hypothetical receptor are under investigation.

A study of the effects of environmental toxins on human placental structure and function is ongoing. The objective of the study is to determine the relationship between heavy metal-induced placental toxicity and the mechanism of heavy metal-induced fetotoxicity. A procedure has been developed for isolating syncytiotrophoblast microvillus membrane vesicles which are derived from the maternal surface of the syncytiotrophoblast. With this system, measurements are being made of the potential effects of mercury and cadmium on the human fetus. It is known that mercury compounds produce toxic prenatal effects in animals and man, and cadmium produces a decrease in size in offspring. The mechanism of action of these compounds remains to be elucidated and the system described will be used to test the hypothesis that modification of placental vesicles may be responsible for these effects.

There is little information available on the effects of halogenated hydrocarbons on the pancreas. It has been determined that both halogenated aliphatic hydrocarbons and halogenated benzenes increase pancreatic juice flow and alter electrolyte and protein concentration of the juice. These are significant effects, but how this influences the overall health of the animal has not yet been determined.

Effects in biliary excretion of PCBs due to TCDD exposure have been observed. Exposure to TCDD impairs excretion of PCBs by this mechanism and thus increases the half-life of PCBs and their metabolites in the body. Since certain metabolites of PCBs are more toxic than the parent compounds, such an impairment in the excretion could lead to increased toxicity. It has also been observed that pregnenolone-16α-carbonitrile or spironolactone treatment reverses the depressed biliary excretion of ouabain in TCDD treated rats. This is the first report of a toxic effect of TCDD which can be reversed by drug treatment.

Research is being conducted to attempt to arrive at an understanding of the neuronal degeneration that occurs following phosphorylation of the enzyme "neurotoxic esterase" (NTE) by certain organophosphorus compounds. The occurrence of this enzyme subsequent to organophosphorus exposure was determined. The finding of NTE in lymphocytes, among other tissues, suggests that there may be considerable scope for further study of a possible immunological component or organophosphate neuropathy. Also, NTE assays could possibly be done in blood lymphocytes as a predictive assay or number of neurotoxicity.

It has been observed that exposure to the PCB, Aroclor 1254 enhances tumor growth and inhibits the development of cellular immunity against the tumor. After 15 weeks of exposure to PCB, animals were injected with MSB-6 tumor and growth rates of the tumor were recorded. Cellular immunity was also assayed periodically. Tumor growth rates and survival time of animals indicated the increased susceptibility to the tumor.

An experimental animal model with which to study drug metabolizing enzyme activity in the skin is being developed. The goal of the project is to assess the impact of selected environmental agents upon these cutaneous enzymes. It has been shown that adult human skin actively responds to topically-applied chemicals by enhanced activity of the carcinogen-metabolizing enzyme, aryl hydrocarbon hydroxylase. It has been shown that skin possesses enzymes capable of activating several chemical moieties into mutagens. In particular, it has been shown that supernatants of neonatal rat skin can activate Tris (the flame-retardant chemical) into a material mutagenic in the Ames system.

Studies are continuing on the mechanism of benzene toxicity mediated through a benzene metabolite. It has been demonstrated that inhibition of benzene metabolism protects animals against toxicity. Using two different strains of mice, C57/B-6 mouse and DBA/SN mouse, it appears that there is a difference in sensitivity to benzene between the two strains. The third strain of mice, the CDL, has been added to the studies and in terms of sensitivity to benzene, the CDL lies between the other strains. Currently, the three strains are being evaluated with regard to covalent binding of benzene metabolites in the organs. It has also been shown that partially-hepatectomized rats were partially protected from the toxic effects of benzene. Evaluation of urinary metabolites show that there was a marked reduction in benzene metabolism in the rats from which most of the liver had been removed.

A study is underway to determine if the presence of halogenated organic pollutants can be generated by enzyme-mediated reactions using naturally-occurring precursors in the presence of chloride and bromide ions. Halogenated compounds, such as chloroform and related halomethanes, halophenols, and related oxide products (biphenyls, phenoxides, benzofurans, and benzodioxines) are potential enzymatic products. The results of these studies have demonstrated that halogenated substances found in the biosphere can be formed enzymatically; thus, it is apparent that substances classified as environmental pollutants may also be biosensitized by living organisms in nature. The experiments undertaken in this project should aid in determining the types of compounds which are precursors to halogenated substances and perhaps to estimate the amount of halocarbons generated metabolically. Such contributions would be extremely useful in the evaluation of toxic levels of halogenated organic compounds.

Recovered frequencies of gene mutations at the dumpy locus with combined treatments of radiation and a chemical mutagen are being studied. The main effort was directed toward determining frequency of dumpy mutations, whole body and mosaic, after treatment with di-methyl-nitrosamine (DEN), with and without gamma radiation. The rationale behind the experiment was the following: DEN produces predominantly fractional mutants, viable as mosaics. If one of the effects of radiation is to produce breakages which can then eliminate one of the products of the first division, then the frequency of mosaics should go down, and the frequency of whole body mutants go up, after such radiation exposure. The results showed a striking effect with DEN in inducing mosaics. There is apparently an interaction of the radiation and the chemical mutagen but it is not completely self-evident and is now being worked on. Moreover, the radiation experiments to produce a new compound of the third chromosome containing all the arms of both homologs has apparently succeeded.

A study is continuing to define the characteristics of distribution, excretion, and metabolism of chlordecone (Kepone) both in humans and in experimental animals. Clinical studies are continuing on the therapeutic use of cholestyramine in reducing the levels of chlordecone in patients who have been exposed to this contaminant. All patients who have received therapy no longer exhibit significant levels of chloredecone in the blood. Two patients continue to exhibit mild neurologic abnormalities which may be permanent. Of particular interest during the past year has been the study of two patients who were poisoned with the organochlorine pesticide, chlordane. The administration of cholestyramine for three days in these patients increased fecal excretion of heptachlor, a metabolic product of chlordane, by three-fold in one patient and by ten-fold in the second. These results support the theory that the present approach to treating patients poisoned with lipophilic toxins need not be confined to chlordecone, nor need it be confined to substances excreted in large quantitites in the bowel. The importance of studies on chlordecone and the establishment of the toxicity and possible carcinogenesis of the compound in humans, lies in the fact that this substance is a widespread contamination of many areas of the southeast.

The mechanism of action of dioxin and a determination of the potential carcinogenicity of the compound is being studied, using cell-free and cell-culture techniques. Male and female Sprague Dawley rats were placed on a diet containing TCDD at 500 parts per trillion for a total of 15 months and were sacrificed. No significant tumor development has been evident in 34 animals necropsied.

Pralidoxime (2-PAM) is the most widely-used alkylphosphate antagonist in the United States. However, this product leads to elevated cyanide levels which may not only be toxic, but can be lethal to man. The toxicological effects of pyridinium aldoxime and alternative antagonists which can circumvent the toxic effect are being investigated. During the past year, 1-methyl-1, 2, cyanopyridinium was isolated and the first description of this labile metabolite in humans is described. Thus, the intermediate for aldoxine metabolism has now been established. It will now be possible to investigate 1-cyanopyridinium chemistry and the relationship of these compounds to the biological metabolites. The significance of these studies lies in the possibility that alternative antagonists can be synthesized where cyanide is not formed as one of the metabolites.

A study is being made to further define the relationship of chronic chemical exposure and glomerulonephritis. The chronic effect of biweekly parenteral injections of carbon tetrachloride, toluene, trichlorethylene and gasoline has been studied by following a number of parameters which suggest glandular injury. The studies suggest that environmental chemicals have the potential to produce cellular injury through chronic parental administration in rats. The mediation of glomerular injury may be related to a direct toxic effect; however, it could also possibly involve fibrinogen, since this substance is frequently noted in the kidneys of the affected animals. The coagulation system is believed to be involved in many forms of human renal disease, leading to renal failure. The significance of these studies lies in the fact that if environmental hydrocarbon exposure is etiologically related to human glomerulonephritis, it would have obvious consequences on preventative measures.

It is known that the mutagenic and carcinogenic activity of most N-nitrosamines requires metabolic activation probably through enzymatic reactivity. The activation is by a mixed function oxidase whose mechanism of action is unknown. Thus, a determination of the structural features or chemical properties of N-nitrosamines may provide information about the enzyme mechanism related to the carcinogenicity of the compounds. Certain N-dinitrosodibenzylamines, with highly acidic hydrogen exhibit no mutagenic or carcinogenic activity, possibly due to the fact that they are not metabolized, or the metabolite lacks activity. In order to distinguish between these two possibilities, the acidic N-dinitrosodibenzylanine was converted chemically to a pseudometabolite, N-nitro-α-acetoxybenzyl-benzylamine. The Ames test showed this compound to be a potent mutagen. This is the first instance of a nitrosamine which was non-mutagenic even on metabolic activation, being converted chemically to a mutagenic derivative. This may be significant in showing that α-acetoxl derivatives are not good models for metabolites, for they may be acylating and not alkylating agents.

Studies to elucidate the physiochemical parameters involved in pulmonary toxicity of compounds may provide a means of predicting lethality, dose-response curves, and theoretically provide a means for deriving safer and less expensive drugs through molecular orientation and engineering. Such has been the basis for a study comparing the chemical structure of heterocycles with their pulmonary toxicity. A correlation between furan pulmonary toxicity with various physiochemical parameters and the preparation of naturally-occurring perilla ketone congeners isoegomaketone and egomaketone for evaluation of pulmonary toxicity was carried out. It was found that liver and kidney toxicity for these agents appears symmetrically distributed about the optimal log p for lung toxicity. These distributions of organ toxicity suggest that given tissue cytochrome P-450 catalytic sites may be optimally programmed for a particular substrate polarity. This hypothesis is consistent with the 4-ipomeanol studies indicating that organ concentrations appear unrelated to irreversible lung-binding with well-known cytochrome P-450 and with evolutionary considerations adapting the enzyme to the more substrate polarity. Results of the studies involving an attempt to delineate the mechanism of furan or heterocycle toxicity in enzymatic bi-activation are consistent with direct electrophilic epoxidation. They tend to support the occurrence of in vivo nucleophilic reactions prior to epoxidation reactions now known to be favored by electron withdrawing substitutes. If the adducts involve the active site, the research could suggest a mechanism for the elusive and rate limiting P-450 substrate binding required for metabolic transformation. These reactions are important to the lung toxicity of these agents.

Rainbow trout which are highly susceptible to the carcinogenic effects of cyclopropenoid fatty acids were fed either a diet containing 50, 150, or 300 ppm of cyclopropenoid fatty acids. Initial efforts were to characterize the effect(s) of various levels of dietary cyclopropenoid fatty acid levels as low as 50 ppm. These alterations were characterized by a reduction in overall unsaturation with a marked increase in levels of saturated and monoenoic fatty acids. Of particular interest was the increase observed in total liver cholesterol. Moreover, this increase appeared to be dose dependent. Total liver cholesterol was 0.22 mg/mg dry lipid at 300 ppm. In contrast, untreated livers showed a value of 0.17 mg/mg dry lipid. Additional studies suggest that cyclopropenoid fatty acids are able to alter plasma membrane structure and that dietary cyclopropenoid fatty acids fed at a low level of 50 ppm causes a significant reduction in cytochrome P-450 levels and epoxide hydrase activity.

Vitamin E and selenium are known to enhance induction of drug metabolism and are both necessary for optimization of this function. Studies have also indicated sex specificity via an involvement of sex hormones in drug metabolism inducement by vitamin E and selenium, and implications for pharmacology and nutrition in humans. These studies are being pursued, using the vitamin E-selenium-deficient chick as a model. Additional studies using this model have demonstrated that toxicity of nitrofurantoin, possibly by oxidative stress, was ameliorated by dietary selenium but not by vitamin E.

A study to characterize and quantify the transport and partitioning of foreign molecules across and within membranes and to characterize the structural changes in the membranes and the physiological activity is underway. A novel spectroscopic method has been developed which can be used to monitor the diffusive transport and local concentrations of chlorinated hydrocarbons in membranes. This method will be useful in elucidating the mechanisms of action of these pesticides.

ORGANIC CHEMICALS

The effects of low-level exposure to polychlorinated biphenyls (PCBs) over extended periods is under investigation. After a period of three years, following the discontinuation of PCB-contaminated diets, adult female Rhesus monkeys are devoid of obvious signs of toxicity, yet their offsprings continue to be small at birth, fail to gain weight as rapidly as controlled infants, are more susceptible to infection, and show learning and behavioral deficits. Aroclor 1016 has been proposed as a "safe" PCB because this particular PCB mixture would be more rapidly metabolized and excreted than other PCB mixtures. However, it was found that with levels of only 0.025 ppm in the diet, nine adult female monkeys had indications of PCB toxicity in their offsprings.

The injurious effects arising from long-term, low-level exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin and the rates of recovery from these effects is being determined. In Rhesus monkeys, it was determined that levels as low as 50 ppt of TCDD in the diet caused injurious effects. The major difficulty is the inability of animals receiving these low levels of TCDD to reproduce. These observations further emphasize the fact that a safe level of TCDD exposure has not yet been established.

The teratological effects of exposures to methylene dichloride (Dichloromethane, DCM) by inhalation before and during gestation at levels of 4,500 ppm is under investigation. Reactivity and habituation to a novel environment were assessed when the offsprings were 5, 10, 15 and 150 days of age. No treatment effects were evident at five days of age; however, beginning at 10 days and continuing through 150 days, treatment effects were evident as reflected by the altered rates of locomotor habituation to the test environment. Treatment differences became more apparent as the test session progressed, and the groups reduced their activity at different rates.

The estrogenicity of Kepone is under study, utilizing the quail oviduct as the experimental model. Higher doses produce complete differentiation of the entire quail reproductive tract, a response similar to that induced to estradiol. However, during such differentiation, even at the lowest concentrations of 10 ppm, abnormalities in the cellular organelles were observed, particularly in the mitochrondria. The reversibility of this effect is now under study.

Studies on the acute effects of the herbicides paraquat and diquat have indicated the most striking effect produced by paraquat to be a rapid and sustained elevation of ATP. This may reflect an increased rate of ATP synthesis to provide energy for repair of cellular damage. It was also observed that the postmortem changes in levels of labile metabolites could be observed by inactivating the tissues utilizing microwave irradiation. Previous studies had utilized a rapid freezing of the lungs and had generated similar values.

The effects of various pesticides on functional development of liver, kidney and lung in perinatal mice and rats is under investigation. The renal toxicity of aminoglycoside gentamicin was determined to be due to active transport of the material as evidenced by dependence on oxygen. It appears that the material is a mitochondrial toxin which exerts harmful effects specifically on the kidney because of the selective uptake by this organ.

Certain chlorinated hydrocarbons manifest estrogenic actions. Therefore, an effort is underway to characterize the biochemical events associated with the estrogenic actions of these compounds and to determine whether certain hydrocarbons affect liver and adrenal monoxygenase-mediated hydroxylation of endogenous substrates, such as prostaglandins. It has been determined that methoxychlor does not block estrogen receptors in the rat uterus. However, incubation of methoxychlor with liver microsomes formed demethylated products which inhibited estrogen binding to uterine-estrogen receptors. An in vitro method was developed which is being refined to allow the facile determination of whether a given estrogen is actually a pro-estrogen. The method involves the incubation of the compound in question with uteri and the presence of liver microsomes and NADPH followed by an examination of the effects of metabolism on the distribution of uterine-estrogen receptors.

The effect of topical exposure to organophosphorus pesticides is being studied by exposing hens to leptophos. It has been determined that the classical gait abnormalities are related to the dose of topical application, similar to the effects seen upon oral administration. Histopathologically, degenerative changes were observed in the myelin and axons in the spinal cords of intoxicated animals. A possible role of hydrolytic enzymes in the mechanism of the induced delayed neurotoxicity is being studied. There is a noted increase in activity of acid phosphatase which correlates with the clincial symptoms observed. These studies indicate that persistent dermal exposure to small doses of these compounds are of potential hazard to humans in industrial and agricultural operations.

It has been observed that the pesticide guthion had an acute toxicity and brain anticholinesterase activity which was potentiated 10 to 20 times in mice pretreated with carboxylesterase inhibitors. This potentiation was surprising since guthion does not contain a carboxylester linkage and because structurally-related organophosphates were much less potentiated. Analytical techniques for the direct determination of guthion are under development to attempt to determine the compound's metabolism.

It has been determined that repeated doses of organophosphate (OP) insecticide at doses which did not cause overt signs of cholinergic excess (toxic manifestations) can cause the development of tolerance. The mechanism of this tolerance is not clear, but there are indications that an insensitivity of cholinergic receptor sites to antagonists may be the cause.

The mechanism responsible for the development of porphyria in animals exposed to hexachlorobenzene (HCB) is not known. There is strong circumstantial evidence that HCB is converted to highly reactive metabolites by mixed function oxidase systems in the liver and other tissues. The covalent binding of these metabolites to protein or other cellular nucleophiles may play a central role in the development of porphyria. Tracer studies are being utilized to identify sites of deposition of metabolized and unmetabolized HCB, and metabolic profiles are being determined.

A multistep fractionation procedure has been developed in which mutagenic activity (toward strain TA1538, in the presence of a PCB-induced microsome preparation), is used to monitor the gradual purification and isolation of mutagenic constituents of commercial (Difco Laboratories) beef extract. The net result is the isolation from the initial beef extract of two apparently homogeneous, mutagenic constituents. Fraction II, which has been the focus of most of the isolation efforts, has a UV absorption maximum at 264 nm and a specific mutagenic activity of 21,000 revertant colonies per ugm, and 74,000 revertant colonies per absorbency unit (at 264 nm). Fraction I has been carried through to the final (HPLC) stage of purification only in preliminary runs, and the weight of the pure material at 274 nm and a specific mutagenic activity of 71,900 revertant colonies per absorbency unit (at 274 nm). The UV absorption spectra of the fractions suggest that the mutagens probably contain aromatic rings, and the acetylation experiment suggests that an amino group is present.

GENETICS

OFFICE OF THE ASSOCIATE DIRECTOR FOR GENETICS
Summary Statement - FY1979

During the past year, the Office of the Associate Director for Genetics has continued to fulfill its role in the Institute's genetic toxicology efforts by providing a focal point and/or coordination function in the following areas of involvement. (1) International programs, (2) National programs, (3) Training courses, (4) Committees, (5) Collaborative Studies, (6) Collaborative Research Programs and (7) Publications and Public Lectures.

International Programs

Under the US-Japan Joint Medical Sciences Program, the Joint Panel on Environmental Mutagenesis and Carcinogenesis held its Eighth Joint Meeting at the East-West Center at the University of Hawaii, July 18-20, 1979. The meeting topic was "Fundamentals of Population Monitoring" and comprised three sessions - Biochemical and Cytogenetic Analysis, Hereditary Disease, and Development of Methods for Identifying High-Risk Populations. The topic was addressed by speakers from both the United States and Japan, including key participation by staff scientists from the University of Hawaii and the Cancer Center of Hawaii.

A group of three Russian geneticists visited the United States January 7-19, 1979, as part of the US-USSR Environmental Protection Agreement. Academician N. P. Dubinin, Dr. V. Filippov and Dr. U. K. Alekperov visited NIEHS, ORNL, LLL, and the University of Michigan to discuss current research efforts in environmental mutagenesis. Discussions of the future direction of the program with the Russians led to an agreement that more emphasis should be placed on training young scientists and the development of collaboration research projects. During the visit of Dr. Y. Altukhov during June 13-24, 1979, a new agreement was developed for collaboration and training in these project areas. This research will not only involve staff scientists at NIEHS and the Institute of General Genetics in Moscow, but other Institutions in both countries as well.

ICPEMC

Two meetings of the Executive Board and Commission of the International Commission for Protection Against Environmental Mutagens and Carcinogens were held during FY79. The first one was held September 18-21, 1978 and the second, April 28-May 3, 1979, in Lausanne, Switzerland, and attended by the ADG who serves as Vice-Chairman. In addition, a planning meeting of the Executive Committee was held on June 16-17, 1979, in Leiden. The main work accomplished in these meetings was the coordination of Committees and Task Group activities. In addition, position papers prepared by both groups were subjected to review and revision. A new Committee was authorized to deal with problems in the area of epidemiology and a new Task Group to set limits of exposure to known mutagens and carcinogens. The first full meeting of members and committees of all five committees will be held in Thoron, France, in September 1979, to permit interaction between members of different committees as well as to further the work of each committee since draft position papers are being developed mainly through correspondence.

National Programs

Both the Associate Director and Assistant to the Associate Director serve as members of the Steering Committee for the EPA's Gene-Tox Program. The program's objective is to evaluate the current status of bioassays in genetic toxicology through an evaluation of information available in the literature.

A workshop attended by thirty experts from several countries was held in Arlington, Virginia, in August 1978, to re-evaluate the published protocol for conducting the Ames Test. A number of recommendations pertaining to protocol, metabolic activation, and analysis and presentation of data resulted from that workshop and were published in Science, Mutation Research, and Environmental Mutagenesis. The recommendations were intended to help standardize the conduct of the test and were aimed primarily at scientists working in testing facilities.

A workshop on Systems to Detect Induction of Aneuploidy by Environmental Mutagens was held in Savannah, Georgia, November 5-8, 1978, to determine (1) the impact of aneuploidy on human health, (2) discuss and assess assay systems for detecting the induction of aneuploidy and (3) stimulate development and validation of aneuploidy assays. It was apparent that induction of aneuploidy is a genetic event which contributes substantially to human genetic disease but which has been largely ignored in mutagenicity assay system development and application. The organization of a collaborative research effort to expedite development and validation of aneuploidy assays was proposed and is presently being pursued.

The role of DNA repair processes in mutation-induction and risk-assessment has become an issue of broad concern. To address this issue a conference on "DNA Repair and Mutagenesis in Eukaryotes" was held in Atlanta, Georgia, June 25-28, 1979. This three-day conference addressed basic scientific questions pertaining to the relation between repair and mutation-induction in eukaryotes as well as differences and similarities in this relation among organisms from fungi to man.

Training

In collaboration with Dr. John W. Drake (LEM), the Associate Director for Genetics developed a graduate level course in Environmental Mutagenesis which was offered in the Genetics curriculum at the University of North Carolina in Chapel Hill during the 1979 Spring Semester.

Committees

The ADG chairs the Subcommittee on Environmental Mutagenesis (DHEW-CCTRP) which has continued to provide a forum for exchange of information and ideas on topics of current interest to government agencies and other groups interested in genetic toxicology. Major topics which were emphasized during the past year were sister-chromatid exchange, mutation monitoring in mammals, computerized data collection, storage and analysis. As Subcommittee Chairman, the ADG has coordinated efforts to obtain funding for the National Academy of Sciences proposed Committee on Chemical Environmental Mutagens.

Collaborative Studies

The MRC/ICI/NIEHS Collaborative Study in Comparative Biological Activity of Paired Compounds has reached the end of the testing phase with all testing to be completed August 31, 1979. This will allow time for investigators and study coordinators to review test data prior to the analysis and evaluation meeting planned for October 1979. Investigators using prokaryotic assays completed testing in February and were convened in Atlanta in March to begin a preliminary evaluation of data. Since chemicals have not been decoded this evaluation could only be made on the basis of code numbers. The meeting furnished a preview of the problems to be encountered in collating and compiling data and provided invaluable experience for planning the larger meeting in October.

The office of the Associate Director for Genetics has assumed an increasing responsibility for coordination and support of this international effort. NIEHS involvement in this study has recently been taken under the Administrative aegis of the National Toxicology Program.

As discussed in the National Programs section, a collaborative study designed to promote the development and application of assays for induced aneuploidy, has been proposed by this office. At present, a source of support is being sought and appropriate investigators are being sought for participation.

Collaborative Research Programs

The analysis of the mutagenic activity of various classes of chemical carcinogens as well as base-line chemical mutagens has continued in wild-type and excision-repair deficient two-component heterokaryons of Neurospora crassa. This work is being performed with the collaboration of Illinois State University, Normal, Illinois. Studies on chemical carcinogens are designed to determine whether those chemicals that are carcinogens produce some characteristic type of genetic damage. In addition, various base-line chemical mutagens which produce characteristic types of lesions (base-pair transitions, frameshift mutations, etc.) are being studied to determine whether similar types of lesions are produced in both eukaryotic and prokaryotic organisms.

In addition, these studies can be used for risk-estimation since they compare the sensitivities of normal strains (wild type) and repair-deficient strains (excision-repair deficient) with regard to the production of genetic damage after exposure to chemical carcinogens and mutagens. Current data show that in Neurospora these mutagens produce the expected lesions in point mutations at the molecular level, but unexpectedly, they also produce multilocus deletions. These latter lesions occur at even higher frequencies in the excision-repair deficient heterokaryon. These studies show that the mechanism of mutation-induction may be different in eukaryotic organisms than in prokaryotes. The marked qualitative and quantitative differences between mutation-induction between wild-type (+/+) and excision-repair deficient (xp/xp) heterokaryons make it desirable to look at heterokaryons which are heterozygous (+/xp) since in the human population such individuals would occur at much higher frequencies than (xp/xp) individuals.

In another research program in collaboration with the Brookhaven National Laboratory (BNL) and ERC-EPA, a higher plant system (Tradescantia) is being developed and used in a mobile laboratory to monitor air pollutants. Exploratory studies of the mutagenicity of air pollutants in high lung cancer incidence counties has continued. In addition, periodic monitoring has continued at the same site in Elizabeth, New Jersey. In the latter case positive test data have been developed continuously throughout the year. Base-line studies have continued to both study the mechanism of flower petal color mutations as well as to develop a more rapid and direct method for scoring mutations using the thin-layer chromatography.

In another area, a test system that has been developed in _Neurospora crassa_ to detect aneuploidy, is being used to screen chemicals for genetic activity. At the workshop on "Test Systems to Detect Aneuploidy" (see above) the summary of chemicals which were found to be positive in this system was used to develop a collaborative study to test their effects in other (higher) eukaryotic organisms. These latter studies will serve to determine the general utility of the Neurospora system as a pre-screen for this class of genetic damage in eukaryotic organisms.

Public Lectures

F. J. de Serres

1. "Application of Short-Term Tests in Toxicological Evaluation of Potentially Toxic Substances", Toxic Substances Control Conference, Washington, DC, December 4-5, 1978.

2. "Problems Associated with the Application of Short-Term Tests for Mutagenicity in Mass-Screening Programs", Acceptance Speech, 10th Annual EMS Award, New Orleans, LA, March 8-12, 1979.

3. "Comparison of the Mutagenic Effects of 2-Aminopurine and Actinomycin-D in Excision-Repair Deficient and Wild-Type Two-Component Heterokaryons of _Neurospora crassa_", 10th Meeting of the Environmental Mutagen Society, New Orleans, LA, March 8-12, 1979.

4. "Evaluation of the Mutagenic Effects of Environmental Chemicals", Tri-Beta Club and Faculty of the University of North Carolina at Greensboro, Greensboro, NC, March 16, 1979.

5. "Organization of the International Commission for Protection Against Environmental Mutagens and Carcinogens", Conference on The Current Status of Short-Term Tests Sponsored by the Toxicology Forum, Washington, DC, May 30-31, 1979.

6. "Rationale and Integration of Short-Term Assays for Carcinogenicity Testing", IARC Workshop on Basic Requirements for Tests to Assess the Possible Carcinogenicity of Environmental Chemicals, Hannover, Federal Republic of Germany, June 4-9, 1979.

7. "Mutation Induction in Repair Deficient Strains in Neurospora", Conference on DNA Repair and Mutagenesis in Eukaryotes, Atlanta, GA, June 25-28, 1979.

8. "Detection of Gene Mutations in *Neurospora crassa*", Conference on Short-Term Tests for Chemical Carcinogens, Vancouver, B.C., Canada, August 22-24, 1979.

OFFICE OF HEALTH HAZARD ASSESSMENT

OFFICE OF HEALTH HAZARD ASSESSMENT
Summary Statement

This office is concerned with the evaluation of human health hazards mainly due to chemical factors. Since it was realized that many chemical products may be responsible on long-term low-level exposure for some of the most dreaded diseases such as cancer, neutotoxicity, sterility, or genetic defects, it has become an order of high priority to detect these toxic chemicals, to understand their potential for disaster, and to help protect the public.

To make correct health hazard assessment requires a more quantitative evaluation of the toxic parameters. For all chemicals there appear to exist no-effect levels, but for those that tend to accumulate in the biosphere, special consideration of dose/response data is necessary. Where synergistic effects can be anticipated, greater attention needs to be paid to these situations wherever they may occur.

One of the basic programs of this office is the compilation of a document on data obtained from the literature on human disease conditions for each organ and tissue to be correlated with exposure data to chemicals and to establish a potential cause effect relationship by a search of laboratory data.

Another task is bringing together all biochemical mechanisms in mammalian systems which affect toxic chemicals and can alter or prevent the adverse results of exposure to these toxic chemicals. It deals with multiple pathways of metabolism and modification of the pathways by nutritional or other environmental factors which might influence the absorption, retention, activation or deactivation, and final elimination of the chemical.

Importance is also attached to the problem of overloading of metabolic pathways and creation of unusual mechanisms of disposal of chemicals, an issue of importance in extrapolation from high to low dose levels. The true impact of DNA repair mechanisms in preventing permanent damage to DNA also needs exploration. Finally, genetic factors must be considered as they may play an often overlooked important role in health hazard assessment and extrapolation from animal to human.

This office continued to supply manpower towards the implementation of the Toxic Substances Control Act. Drs. Falk and Piver remained as member and alternate on the Interagency Committee for Selection of Substances for Testing. In April they were relieved of that demanding activity, which was taken over by Dr. Bates. In recognition of his contributions to this task, Dr. Piver was given the NIH Directors award. This award also recognized his contributions to the World Health Organization and the United Nations Environmental Program. Dr. Damstra continues her participation on the Interagency Toxic

Substances Data Committee to design and coordinate an effective system for information retrieval of toxic substances submitted to EPA under the Toxic Substances Control Act.

Dr. Damstra also serves as NIEHS representative on the Toxicology Information Subcommittee of the HEW Committee to Coordinate Toxicology and Related Programs. She also serves on the TIS Task Group for the Laboratory Data Bank, an online computer system providing comparative pathological data on laboratory animals of different strains. Besides membership on the Chemical Substances Information Network Subcommittee, she has recently become a member of the Interagency Response to Chemical Crises Committee.

Collaboration with the World Health Organization and the United Nations Environmental Program continued unabridged. Dr. Falk participated on Interagency meetings on the implementation of the WHO Environmental Health Program to be activated in a new form on the National level, coordinated by WHO. He also attended the third meeting of the Scientific Advisory Committee for the United Nations Environment Programme's International Register of Potentially Toxic Chemicals in Geneva, Switzerland, December 11-15, 1978. He also represented WHO at the OECD/EPA Good Laboratory Practices Meeting in Washington. Dr. Piver attended two meetings in Geneva, Switzerland, sponsored by UNEP on Environmental Health Aspect of Chemical Industrial Processes, for which he had prepared a major background document. Dr. Falk attended the International Atomic Energy Agency's (IAEA) advisory group meeting on Comparative Health Impacts of Nuclear and Alternative Sources of Energy in Triest, Italy. On that same topic Dr. Jurgelski attended a workshop on the Health and Environmental Effects of Coal Gasification and Liquefaction, held in Washington, D. C.

Dr. Posner continued his participation in the Interagency Committee for Stratospheric Ozone Protection and became actively involved in the paraquat spraying of marihuana problem, assessing health effects of alternatives and assessing the effectiveness of potential marker compounds as part of a DHEW ad hoc committee.

For the Interagency Task Force on Cancer, Lung and Heart Disease, Drs. Jurgelski, Damstra, Falk, and Piver prepared a document assessing the environmental exposure, and metabolic parameters of a number of very important industrial and environmental air pollutants, namely benzene, toluene, carbon disulfide, and methylene chloride. These documents serve to explore new ways in which information of importance to many agencies, the interested public, and scientists would be brought before them in a timely fashion to inform, alert, and prepare for potential action.

The staff of OHHA continued to collaborate under guidance from CDC in giving constructive criticism to documents prepared by other agencies outside or inside DHEW on the topic of environmental/occupational health hazards. The staff also reviewed grant applications received by USDI on research in the area of water pollution and purification. All these activities which proved frequently very time consuming were recognized for their value and a group award was given to members of OHHA involved in this activity by the Director, NIEHS. The recipients of the award were Drs. Piver, Damstra, Jurgelski, Posner, and Mrs. Edmonds.

Dr. Falk remains a member of the International Joint Commission's Committee on the Assessment of Health Effects of Great Lake Water Quality and is involved together with Dr. Damstra in the evaluation of 450 chemicals detected in the Great Lakes for their potential carcinogenic and neurotoxic properties. This information will be combined with other evaluations regarding potential toxicity and lead to in-depth studies on quantitative aspects of these observed contaminations of the Great Lakes.

The Ad Hoc Advisory Committee on Toxicity of Exposure to Agent Orange to the Veterans' Administration of which Dr. Falk has been a member has discontinued its deliberations at the end of 1978 in order to become a properly constituted committee. The interagency committee on the carcinogenicity of nitrites called for by FDA of which Dr. Falk is a member has decided to call for a complete pathological re-examination of the findings and will shortly review the conclusions. An Ad Hoc Committee on the Toxicity and Carcinogenicity of Beryllium Compounds was requested by the Secretary DHEW, and Dr. Falk was a member of the group assessing the evidence of carcinogenicity of beryllium compounds to laboratory animals and epidemiological evidence of the carcinogenicity to humans exposed industrially to beryllium compounds.

Dr. Falk was a member of an Interagency Committee on Ethics to help the DOD with the human voluntary exposure problem on safeguards regarding potential exposure to toxic chemicals and the communication of potential risks to their volunteers. Dr. Falk resigned from this Committee in order to reduce travel expenses.

The Subcommittee on Laboratory Chemical Carcinogen Safety Standards of which Dr. Falk is a member is coming to the end of their deliberations after having presented their report to the public and having received valuable comments for incorporation into the final document. The committee will expire the end of 1979.

On invitation Dr. Falk attended a conference on World Economic and Industrial Development and World Health at the Poznan University of Poland, September 7-9 to present a paper on Threshold Effects Involving Industrial Pollutants. He also attended a meeting called for by the New York State Health Department to discuss health effects monitoring and remedial action in connection with the exposure of people to the hazardous chemicals in the Love Canal dump. In January he participated in the Interagency meeting of the National Toxicological Program to crystallize the needs of that program in sequential fashion covering all areas of toxicology and the methodologies to employ or develop. As a new member he attended the advisory committee meeting to review the health program of the Brookhaven National Laboratories of the Associated Universities, Inc.

At the 63rd Annual Meeting of the Federation of American Societies for Experimental Biology, Dallas, Texas, Dr. Falk gave a paper on Extrapolating Carcinogenesis Data from Animals to Humans in a Symposium: Extrapolation of Laboratory Toxicity Data to Man: Factors Influencing the Dose-Toxic Response Relationship. Dr. Posner attended the joint meeting of the American Chemical Society and the Chemical Society of Japan in Honolulu, Hawaii, to present a paper entitled, "Chemical Causes of Prenatal Maldevelopment.

Dr. Jurgelski attended the XIIth International Cancer Congress in Buenos Aires, Argentina, making a presentation. He also attended the FASEB meeting in Dallas, Texas, where he presented his findings in a poster session. He participated in a Conference on Water Quality Criteria called for by EPA at New Orleans and attended the meeting of the Society of Toxicology in the same city.

Dr. Piver participated in the NIEHS program evaluation workshop at Boston, attended a Laboratory Chemical Waste Destructor Evaluation meeting at Bartlesville, Oklahoma, and a NIEHS planning committee on Hazardous Wastes in Washington, D. C. He also serves as member of the NIEHS Safety Committee and as member of the Advisory Board for SciQuest Magazine to help plan the publications aimed at High School students.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 20001-07 OHHA

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Variations in the Response of Rats to Porphyrogenic Chemicals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Hans L. Falk	Assoc. Dir., OHHA	OHHA	NIEHS
OTHER:	Sandra Jordan	Technician	OHHA	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
None

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N.C. 27709

TOTAL MANYEARS: 1.1	PROFESSIONAL: 0.1	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Identification of urinary porphyrin metabolites from normal rats as well as those pretreated with porphyrogenic chemicals such as hexachlorobenzene or allylisopropylacetamide. Among the treated animals those that respond with porphyria are compared to those that do not in spite of identical treatment.

So far a qualitative study has been carried out on urine samples over the two-year period of observation and differences in the metabolites with age and treatment are being compiled.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Urine samples are from female rats (Charles River CD). The experimental animals received their first dose of hexachlorobenzene (HCB) (0.2% in rat food) at 28 days of age. Feeding HCB continued for three weeks. Porphyric animals received no more HCB. Non-responding animals received one or two additional doses of HCB. All experimental animals at 18 months of age received doses of allylisopropylacetamide (AIA) at intervals either in rat food (0.2%) or in cottonseed oil by gavage (100 mg/kg). Urine was collected at weekly intervals from control and experimental rats. They were stored at -20°C in a freezer. For analysis the pH was adjusted to 4 and the porphyrins were adsorbed on talc. The talc was then freeze-dried and the porphyrins were esterified. The methyl esters were extracted with chloroform, flash-evaporated and made up to volume in chloroform. Aliquots were spotted on silica gel thin layer chromatography plates and developed. Spots were detected by long wave ultraviolet light irradiation observing the red fluorescence and compared to porphyrin standards and to data given in the literature.

MAJOR FINDINGS AND PROPOSED COURSE: In untreated rats the major metabolite was coproporphyrin and a minor metabolite detected by its fluorescence was so far not identified. With age the urine of these control animals showed the presence of another porphyrin metabolite which has not been identified.

In porphyric rats, following treatment with hexachlorobenzene which remains in the animals and thus produces prolonged porphyria, the major metabolites are, in order of decreasing intensity, porphyrins with 8 carboxylic acid groups, 7, then 6 carboxylic acid groups and coproporphyrin. This gradation in intensity is maintained through the period of intense porphyria. With the decline in porphyria the metabolites decrease to below normal levels. Even coproporphyrin becomes very weak as measured by fluorescence.

With allylisopropylacetamide, which produces a short-lived acute porphyria, the urinary metabolites are similar to those mentioned above. Coproporphyrin may play a less important role.

Animals exposed to porphyrogenic chemicals that did not respond with porphyria showed porphyrin metabolites in the urine as did the untreated controls.

Following characterization of the porphyrin metabolites in rat urine quantitation of each metabolite by fluorescence or absorption spectroscopy will be carried out. When completed the use of radioactive delta-aminolevulinic acid given to rats during hexachlorobenzene exposure should allow further understanding of the metabolic events in the responding animals as compared to the non-responding rats. Administration of small amounts of radioactive hexachlorobenzene may be necessary to assure that lack of availability of the porphyrogenic chemical is not the reason for the lack of porphyria in the unresponsive animals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Variations in human response to environmental chemicals are always observed but such drastic variations have not been followed in laboratory studies to any extent. The very marked variation in the response to porphyrogenic chemicals may serve as a model to study differences in toxicologic responses and to determine the factors responsible for them.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 20002-07 OHHA

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Technology Forecasting and Technology Assessment

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Warren T. Piver	Chemical Engineer	OHHA	NIEHS
OTHER:	Hans L. Falk	Assoc. Dir. for OHHA	OHHA	NIEHS
	Herbert S. Posner	Pharmacologist	OHHA	NIEHS
	Terri Damstra	Biochemist	OHHA	NIEHS

COOPERATING UNITS (if any)

Institute-wide

LAB/BRANCH
None

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goals of this program are to develop and apply techniques for technology forecasting and technology assessment for the chemical process industries that would provide guidance in setting research priorities for environmental chemicals.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: To accomplish these purposes, synthetic organic chemicals are discussed in the context of their method(s) of synthesis, normal commercial use patterns, and methods of disposal. With this approach, it is possible to examine and explain the origin of contaminants and by-products in intermediate and end-product chemicals. Besides information on environmental entry of organic chemicals during production, use, and disposal, data are collected on environmental transport and transformation, the potential for bioaccumulation, and the toxicity of the chemical.

Assembling this information on individual chemicals accomplishes the following: (1) provides a better understanding of origins of pollutants; (2) identifies gaps in knowledge; (3) identifies specific industrial and commercial processes and operations requiring pollutant monitoring and possible equipment design modifications; (4) identifies trends in rates of production, rates of substitution of products, and the impact of Federal Legislation and programs on chemical development; and (5) provides additional data on relationships between chemical structure and biological activity and environmental effects.

MAJOR FINDINGS AND PROPOSED COURSE: Data on the entry, movement, bioaccumulation and toxicity of commercially important organic chemicals have been assembled into a report. This report started with four major petrochemical feedstocks and followed these chemicals through their synthesis into end-product chemicals. This review identified many gaps in knowledge on toxicity and control of exposure and release to the environment of many commercially important organic chemicals. In addition, it identified the origin of trace amounts of other chemicals in the primary chemical product that were present as contaminants or as intentional additives.

By use of the methods of product substitution, it was possible to forecast the approximate time that new chemicals would take over substantial percentages of the market. In this area of product substitution, the emphasis is on those chemicals that are similar in molecular structure to chemicals with known biological activity. This analysis was carried out for vinyl bromide substituting for vinyl chloride in modacrylic fibers.

The proposed course is to continue this analysis of product substitution and identify more accurately those factors that influence the rate of substitution of new chemical products, particularly the influence of Federal legislation. In addition, methods are being developed to calculate electronic energy levels for organic chemicals. This will be coupled with pattern recognition techniques to determine if possible correlations exist between electronic energy level patterns and biological activity. When chemicals are grouped by similar structure, such a correlation can be used to assess the potential biological activity of new chemicals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These activities will continue to provide necessary information about the entry of commercially important organic chemicals into the environment and gaps in knowledge with regard to toxicity, bioaccumulation and environmental transport and transformation. Such information provides a firmer justification for toxicity evaluation programs within the Institute, and facilitates the performance of duties as an alternate member of the Interagency Testing Committee of TOSCA.

PUBLICATIONS

Piver, W. T.: Environmental Health Problems Associated with the Manufacture and Use of Synthetic Organic Chemicals. Prepared for the World Health Organization -- Report HCS/78.2, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20003-06 OHHA

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Preventive Surveillance of Environmental Chemicals for Toxic Potential

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Herbert S. Posner	Pharmacologist	OHHA	NIEHS
OTHER:	Hans L. Falk	Assoc. Dir. for OHHA	OHHA	NIEHS
	Raymond E. Shapiro	Asst. Dir. Toxicol. Coord.	OD	NIEHS
	Alan G. E. Wilson	Visiting Associate	LPK	NIEHS
	Douglas B. Walters	Tech. Program Mgr.	ECB	NIEHS
	Hazel B. Matthews	Research Chemist	LPK	NIEHS
	Linda Markley	Summer Aide	OHHA	NIEHS

COOPERATING UNITS (if any)
Interagency Study Group on Health Considerations of the Use of Paraquat and 2,4-D to Eradicate Marihuana; Interagency Committee on Stratospheric Ozone Protection

LAB/BRANCH
OD, LPK, ECB

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.3	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project uses a variety of techniques for early awareness and attempted reduction of chemical- and physical-agent-mediated health hazards. Some of the projects this year were as follows. The 262 compounds with a vinyl halide subgroup, of commercial, contaminant and natural product significance, were sorted into several subcategories for comparison with pharmacologic and toxicologic information. Information regarding the safe use of herbicides for the control of marihuana was investigated in coordination with others at NIEHS and in an interagency study group. This has involved mainly the present use of paraquat or the potential use of a 2,4-D ester or another compound as herbicides, and of (+)-limonine dimercaptan and other compounds as an odorant marker. The literature is being monitored with regard to methanol toxicity, the uses and safety of aminimide compounds and health effects of changes in stratospheric ozone, the latter due to anthropogenic influences. Several committees are served, documents from other Government agencies are reviewed and requests for information are answered.

PROJECT DESCRIPTION

METHODS EMPLOYED: Searching of the literature, consultations with appropriate individuals, consideration of structure-activity relationships, preparation of reports, discussions with those who might assist in further laboratory or theoretical considerations, and preparation of manuscripts for publication; reviews of documents and manuscripts and response to letters as requested.

MAJOR FINDINGS AND PROPOSED COURSE:

Compounds with a vinyl halide substructure: The list of 262 compounds with a vinyl halide type substructure, that have been used commercially or are present as contaminants in other products or are found in the natural environment, have been sorted into several groups based upon further aspects of their structures. It is hoped to gather toxicologic data for some of the compounds of each group to prepare a structure-activity analysis.

Methanol: The proposed uses of methanol are being monitored to determine if there will be additional large quantity uses in the generally open environment. This occurrence could cause a substantially greater possibility of human exposure to the methanol vapor or to it by ingestion (due to its slight inebriation effect). Previous proposals for the use of methanol as an alternative vehicular fuel, either alone or in mixture with gasoline, are not as prominent any more. Rather, ethanol seems to be gaining in popularity to be the alcohol portion of "gasohol," a 10-15% solution of the alcohol with gasoline. Ethanol does not share some of the more severe, acute and sometimes irreversible hazards seen with methanol.

Paraquat and a potential chemical marker for its presence: About a year ago it became known that marihuana in Mexico was being sprayed with a solution of the herbicide, paraquat, to destroy the crop in the field. A large number of meetings have now been held for many of which I have been a participant. At first, potential alternative herbicides were considered. Then potential color and odorant markers were considered that would indicate to a user that their marihuana was contaminated. Extensive library searching was done with regard to the health hazards and chemistry of: paraquat, other potential herbicides and potential color and odorant markers. Internal documents have been prepared and the Environmental Impact Statement prepared by the Department of State was reviewed twice. A contract was established with the Research Triangle Institute to determine more accurately the amount of paraquat that comes through a cigarette smoking machine with the marihuana cigarettes. This is discussed in the report by Dr. Raymond E. Shapiro, Assistant to the Director for Toxicology Coordination. Also Dr. Alan G. E. Wilson, Dr. Douglas B. Walters, Dr. Hazel B. Matthews, and Miss Deborah Cohen have been involved with this project.

Stratopsheric modification: The Interagency Committee on Stratospheric Ozone Protection (ICSOP) was established as a successor to the previous Federal Task Force on Inadvertent Modification of the Stratosphere (IMOS) and its subgroup, the Biological and Climatic Effects Research (BACER), on both of which I served. I will participate in the Human Health Effects subgroup of ICSOP as the NIEHS representative. The purpose of the group is to coordinate research in the field to clarify questions of the hazards of potential alteration of the concentration of stratospheric ozone in coming years.

Aminimide compounds: Reports on the synthesis and chemical reactions of compounds containing a $\bar{N}$-$\bar{N}$ group continue to be active. In addition to the broad spectrum antimicrobial activities mentioned in the two previous annual reports, there are now reports of antineoplastic activity in mice and rats and pesticidal activity to mosquito larvae and pupae. Aminimide polymers are being used to deliver herbicides as pendant side chains and to hold compounds like benzo[a]pyrene, 11-deoxycortisol and N-phenylimidazole for affinity chromatography. Many industrial and commercial uses of the compounds are being explored. However, toxicologic and metabolic information for the compounds remains essentially absent from the open literature. This situation deserves to be rectified soon. I will continue to monitor the literature.

Card file of chemical and physical agents considered in NIEHS intramural research: The card file begun and described in the last annual report has been continued. It has been used on request to identify those individuals involved with particular chemical and physical agents.

Other NIEHS and outside activities: Draft documents prepared by several Government agencies were reviewed for their health hazard content. I was one of five in OHHA included in a group award for this activity. Requests for information are answered and committees are served. I have been the NIEHS contact with the North Carolina Academy of Science.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The project identifies areas were research is needed in order to prevent or reduce toxicologic hazard from environmental chemicals or physical agents. It is primarily a program of preventive and early-warning surveillance. It is linked closely to research dealing with the scope of the problem, the mechanisms involved, and preventive measures. It also considers potential means of diagnosis and therapeutics where these are possible.

PUBLICATIONS

Posner, H. S., Damstra, T., and Nriagu, J. O.: Human health effects of lead. In Nriagu, J. O. (Ed.): The Biogeochemistry of Lead in the Environment. Amsterdam, Elsevier, 1978, Chapter 15, pp. 173-223.

Posner, H. S.: Environmental contaminants: General and particular considerations. Assoc. Food Drug Off. Quart. Bull. 43: 134-147, 1979.

Posner, H. S.: Chemical causes of prenatal maldevelopment. American Chemical Society/Chemical Society of Japan Chemical Congress, Div. Chem. Health Safety, Abstr. No. 22, 1979.

Posner, H. S.: Chemical causes of prenatal maldevelopment. In Walters, D. B. (Ed.): The Safe Handling of Chemical Carcinogens, Mutagens and Teratogens: The Chemist's Viewpoint. Ann Arbor, Ann Arbor Science Publishers, Inc. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 20004-05 OHHA

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Surveillance of Potential Environmental Health Hazards

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Terri Damstra	Biochemist	OHHA	NIEHS
OTHER:	Hans L. Falk	Assoc. Dir. for OHHA	OHHA	NIEHS
	Warren T. Piver	Chemical Engineer	OHHA	NIEHS
	Herbert S. Posner	Pharmacologist	OHHA	NIEHS
	Jane Ellen Simmons	Biologist	OHHA	NIEHS

COOPERATING UNITS (if any)

Institute-wide

LAB/BRANCH
None

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MANYEARS: 1.2	PROFESSIONAL: 1.0	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this long-term project are: (1) to compile an index of associations between organ system diseases and symptoms in humans and exposure to environmental and occupational chemicals; and (2) to assess the practicality and validity of using neurological and behavioral tests as early indicators of toxicity and as surveillance "tools".

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Preparation of an open-ended data file, which is continually updated via constant literature surveillance along with computer access to various bibliographic and numerical data banks. Membership on committees; attendance at meetings; reviews of documents and manuscripts; evaluation of computerized data files; preparation of reports and monographs; and consultation with scientists from other government agencies, industry, and academia.

MAJOR FINDINGS AND PROPOSED COURSE:

The diseases, syndromes, and symptoms associated with several organ systems have been identified and indexed. A preliminary list of associations between exposure to environmental and occupational chemicals and human nervous system, respiratory system, and cardiovascular system diseases has been compiled. Specific target organ toxicity may result from exposure to a variety of chemical agents.

In some instances cause/effect relationships are clearly established, whereas the evidence for other associations is limited to a few anecdotal case reports. Many symptoms are nonspecific and can be attributed to numerous factors. It is therefore difficult to correlate such symptoms with chemical causes unless some preliminary warning of the possible hazard has been given. Nevertheless, these symptoms may be the first sign of intoxication with environmental chemicals.

There is at present a lack of rational criteria for selecting suitable experimental procedures to determine toxic neurobehavioral effects in both animal test systems and human populations. Efforts to compile an index of toxicological symptoms and diseases and to evaluate testing procedures for assessing neurobehavioral toxicity will continue.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A compilation of associations between target organ toxicity and exposures to chemicals will aid the Institute in making a proper assessment of potential health hazards and in identifying the research programs necessary before such assessments can be made. An index of toxicological symptoms and diseases will also assist in the differential diagnosis of diseases considered likely to be of toxicological origin.

PUBLICATIONS

Posner, H. S., Damstra, T., and Nriagu, J. O.: Human health effects of lead. In Nriagu, J. O. (Ed.): The Biogeochemistry of Lead in the Environment. Amsterdam, Elsevier, 1978, Chapter 15, pp. 173-223.

Damstra, Terri: Neurochemistry and Toxicology: Overview. Environ. Hlth. Perspec. 26: 121-124, 1978.

Damstra, Terri: Environmental chemicals and nervous system dysfunction. *Yale J. Biol. Med.* 51: 457-468, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 20007-02 OHHA

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Identification and Evaluation of Environmental Health Hazards: Chemicals and Chemical Carcinogens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	William Jurgelski, Jr.	Medical Officer	OHHA	NIEHS
OTHER:	Hans L. Falk	Assoc. Dir. for OHHA	OHHA	NIEHS

COOPERATING UNITS (if any)
Institute-wide

LAB/BRANCH
None

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
The objective of this project is to identify and evaluate real and potential health hazards in the environment with emphasis on chemicals both as toxicants and as carcinogens.

PROJECT DESCRIPTION

METHODS EMPLOYED: Review of existing data in the open literature and in unpublished manuscripts; preparation of reports and monographs; consultation with scientists from other governmental agencies, industry, and academia, both domestic and foreign.

MAJOR FINDINGS AND PROPOSED COURSE: 1. The principal investigator prepared a review on the metabolism of benzene and toluene for the Task Force on Environmental Cancer and Heart and Lung Disease. The review yielded the following recommendations for future research on these two chemicals: a. Studies are needed to identify the best animal model for benzene metabolism in the human; b. The metabolism, disposition, and time course-concentrations of benzene and its metabolites in the bone marrow during benzene intoxication should be further defined and characterized; c. Once the compounds present in bone marrow at the time of benzene intoxication are fully identified and quantitated, their effects on bone marrow activity should be determined in an effort to identify the active metabolite(s); d. Further and more detailed studies should be conducted on the synergistic and inhibitory effects on benzene metabolism of chemicals to which humans may be exposed coincident with exposure to benzene; e. A precise metabolic index for the level of human exposure to toluene should be developed. The currently used rate of hippuric acid excretion is not a quantitative measure of exposure; f. The pharmacokinetics of toluene require further definition; g. Further and more detailed studies should be conducted on the synergistic and inhibitory effects of toluene on other chemicals to which humans may be exposed coincident with exposure to toluene.

2. The Principal Investigator was a member of the Workshop on the Health and Environmental Effects of Coal Gasification and Liquefaction, co-sponsored by EPA, NIEHS, and MITRE Corporation held in Leesburg, Virginia in August 1978. He was responsible for preparation of the recommendations for research on systemic toxicity of coal conversion materials. The report will be prepared for distribution to interested parties.

The Principal Investigator also attended a Conference on Water Quality Criteria sponsored by EPA and held in New Orleans in March 1979.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Critical reviews and periodic reevaluations of the type described provide (1) a basis for a more balanced assessment of the risks vs. benefits associated with potential and existing health hazards and (2) reveal those deficiencies and inconsistencies in the available scientific information which require further research and new approaches.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 20008-02 OHHA

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Marsupial Neonate as a Model for the Identification and Evaluation of Environmental Toxicants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	William Jurgelski, Jr.	Medical Officer	OHHA	NIEHS
OTHER:	Pearlie M. Hudson	Physical Sci. Tech.	LET	NIEHS
	Hans L. Falk	Assoc. Dir. for OHHA	OHHA	NIEHS
	L. E. Zimmerman	Ophthalmic Pathologist	AFIP	
	J. M. Henry	Neuropathologist	AFIP	
	N. Palmer	Renal Pathologist	The Childrens Orthopedic Hospital & Med Center Seattle, Washington	
	S. Hoffman	Oral Pathologist	Univ. Alabama	

COOPERATING UNITS (if any)

Medical Arts and Photography, NIH

LAB/BRANCH
Office of Health Hazard Assessment

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project seeks to develop the opossum Didelphis virginiana as a biomedical model by (1) characterizing the normal neonatal and developing opossum anatomically and physiologically and (2) determining the pathophysiologic response of these animals to selected environmental toxins and carcinogens.

PROJECT DESCRIPTION

METHODS EMPLOYED: Special histologic techniques, light and electron microscopy.

MAJOR FINDINGS AND PROPOSED COURSE:

(1) Opossum Film. Completion of the opossum films (entitled: "The American Opossum as a Biomedical Model". Part I: "The Neonatal and Developing Opossum" and Part II: "The Adult Opossum") continues to be delayed due to unavailability of resources. NIEHS management previously indicated that funds would not be released for the purpose of completing this work until the carcinogenesis work was complete. A request for funds is in preparation.

(2) Carcinogenesis Study. (a) Work continues in an attempt to complete the characterization of the lesions induced in developing opossums with ethylnitrosourea (100 mg/kg).

(b) Collaborative Studies and Consultation. The principal investigator is collaborating in several studies utilizing the opossum, being conducted or planned at other institutions.

(1) In collaboration with Dr. Bernd Hamprecht of the Max Planck Institute of Biochemistry in Munich, West Germany, a small opossum breeding colony has been successfully established with the objective of reproducing the brain (ganglioglioma) and eye (teratoid medulloepithelioma) neoplasms induced with ethyl nitrosourea. (Please see Annual Reports for FY 74 and 75.) Tissue from these two tumor types will be (a) frozen as part of a tumor tissue bank, (b) innoculated into nude mice, and (c) placed in tissue culture for morphological, biochemical and electrophysiological studies of neoplastic neurons in vitro.

(2) The principal investigator functions as a consultant to the Laboratory of Gastrointestinal Physiopathology, Department of Medicine, University of Lenven, (Drs. J. Jaspens and G. Vantrappen), Belgium in a study of the physiology of peristaltic contraction in the esophagus of the opossum.

(3) The principal investigator is a consultant to the Netherlands Central Institute for Brain Research, Amsterdam, The Netherlands, in a comparative morphologic study of the opossum pineal gland (Dr. P. Pevet) and work on nerve regeneration (Dr. R. Baber).

(4) The principal investigator is collaborating in establishing a research program to reproduce embryonal tumors of the jaw using the opossum neonate by the Department of Pathology, University of Alabama Medical School, Birmingham, Alabama (Dr. S. Hoffman).

(5) The principal investigator is collaborating in establishing a research project involving the induction of retinoblastic neoplasms in the neonatal opossum at the Eye Institute of Retina Foundation, Harvard Medical School (Dr. Mukai).

(c) Contract Studies. Attempts to re-establish the opossum carcinogenesis study with NCI support did not materialize due to a change in administration at NCI. The current approach is to establish the world-wide need for a marsupial center by identifying present and potential beneficiaries of such a center.

The proposed course of this work is as follows:

(1) Carcinogenesis Study. Histologic preparation of tissues and histopathologic interpretation and classification of lesions will continue as resources allow.

(2) Opossum Films. Completion of the films will depend on the availability of pregnant opossums and technical and fiscal support.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The opossum model provides an opportunity to directly evaluate the relationship between susceptibility to embryonal carcinogenesis and differentiation of the target tissue. The model may also be of value in exploring the apparent interrelationship among oncogenesis, teratogenesis, and mutagenesis in the absence of the major handicap intrinsic to the eutherian animals; namely, the impossibility of distinguishing direct carcinogen induced teratologic/mutagenic changes from identical lesions which are the indirect result of adverse physical and physiologic effects of the carcinogen on the maternal and fetoplacental unit. In the semi-embryonic, semi-fetal opossum orally or parenterally exposed to a carcinogen, mutations and malformations can only be either carcinogen induced or spontaneous.

PUBLICATIONS

Jurgelski, W. Jr., Hudson, P. M., Falk, H. L., Zimmerman, L. E., Henry, J. M. and Palmer, N. F.: Experimentally Induced Embryonal Neoplasms in the Opossum. Some Aspects of Tumor Biology in Comparison to Analagous Pediatric Neoplasms in Man. In Severi, L. (Ed.): Tumors of Early Life in Man and Animals. Monteluce, Italy, Perugia Quadrennial International Conference on Cancer, 1978.

Jurgelski, W. Jr.: The Developing Marsupial as an Animal Model in Space Biology. In Message, M. (Ed.): Proceedings of the Conference on Cell and Molecular Biology in Space. New York, Elsevier/North Holland, 1979 (In press).

Jurgelski, W. Jr.: Tissue Differentiation and Susceptibility to Embryonal Tumor Induction by Ethyl Nitrosourea in the Opossum. In Rice, J. M. (Ed.): Proceedings of the Conference on Perinatal Carcinogenesis. Washington, D. C., Govt. Print. Off., 1979 (In press).

Vandeberg, J. L., Cooper, D. W. and Jurgelski, W. Jr.: Sex chromosome dosage compensation in marsupials other than kangaroos: Preliminary evidence from PGK-A, G6PD and alpha GAL variants. Isoenzyme Bulletin, 1979 (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 20009-01 OHHA

PERIOD COVERED
October 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Identification of Potential Environmental Health Hazards

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jean Bernheim	Microbiologist	OHHA	NIEHS
OTHER:	Hans L. Falk	Assoc. Dir. for OHHA	OHHA	NIEHS
	William Jurgelski, Jr.	Medical Officer	OHHA	NIEHS
	Terri Damstra	Biochemist	OHHA	NIEHS
	Warren T. Piver	Chemical Engineer	OHHA	NIEHS

COOPERATING UNITS (if any)

Institute-wide

LAB/BRANCH
None

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.8	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aim is to identify and evaluate potential health hazards in the environment from exposure to chemicals by an in-depth search of the literature. The mechanism of action of the toxicant is the ultimate goal of this endeavor.

PROJECT DESCRIPTION

METHODS EMPLOYED: The open literature is consulted for identification of chemical structures that are associated with certain biological or toxicological effects. This information is collected, classified on the basis of chemical structure and completely documented. The report may be prepared for use as background documentation or serve the staff of OHHA in their function.

MAJOR FINDINGS AND PROPOSED COURSE:

Substituted aromatic amines and derivatives: A correlation was made between chemical structure and carcinogenic activity (potency, target organ specificity) in two species, two sexes based largely on information derived from the NCI bioassay data collection. This compilation is helpful in suggesting carcinogenic activity in closely related chemicals which have not been on test.

Human chromosome breakage and exposure to chemicals: The literature contains information on that topic which needs clarification and assessment. Different types of chromosomal abnormalities and specific chemical exposures have been searched for.

DNA repair inhibitors: This search of the literature will have to continue for some time as the topic becomes of greater interest to scientists and more data will be published. A distinction must be observed between general protoplasmic poisons and specific inhibitors of specific DNA repair enzymes. This is an important area which is only recently activated.

Metabolism of specific chemicals in animals and humans: The literature was searched for metabolic data on specific chemicals of interest, i.e. benzene, toluene, carbon disulfide, and methylene chloride to understand the toxicity observed in animals and humans as reflected by the metabolic processes involved in handling these chemicals in the organisms under study.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Information available in the literature often times can give us clues on potential hazards that may be anticipated on exposure to new chemicals which are bearing close relationship to other better known chemicals. The mistake of making too sweeping generalizations as has often been done in the past has tended to discredit this structure/activity correlation, but when done with proper care and limitation it is a very good tool for hazard assessment.

BATTELLE PACIFIC NORTHWEST LABORATORIES - Richland, Washington
(NIH-NIEHS-78-2150)

TITLE: Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants

CONTRACTOR'S PROJECT DIRECTOR: R. E. Wildung, Ph.D.

PROJECT OFFICER (NIEHS): Warren T. Piver, Ph.D., Chemical Engineer,
Office of Health Hazard Assessment

DATE CONTRACT INITIATED: October 1, 1978

CURRENT ANNUAL LEVEL: $104,680

PROJECT DESCRIPTION

OBJECTIVES: The objectives of these investigations are to determine using advanced radiotracer, spectroscopic, and chromatographic techniques: (1) the rate and extent of uptake of metals from soils by representative grass and broadleafed plants; (2) the translocation of metals in plants; (3) the effect of metals on soil microbiota and soil microbial processes; (4) the nature of metal bond types and chemical forms of metal metabolites in plant and microbial tissues; and (5) the potential for soil formation of organometal complexes, and subsequent uptake of these compounds by plants. Implicit in these objectives are the development of suitable methods for determining the chemical forms of metals in soils, plants, and microbial tissues.

The metals are nickel, cadmium, chromium, and thallium, and the plants are soybeans.

METHODS EMPLOYED: Ritzville soil was thoroughly mixed with labeled and unlabeled isotopes. A split-root (soil/nutrient solution) method was employed to grow soybeans to maturity in the metal containing soils. The plants were cultured in a growth chamber under constant light (14 hr light; 10 hr dark), temperature (27°C light' 20°C dark) and humidity (40-45%). At maturity the plant tops and roots were harvested (133 and 142 days from planting). Plants were separated by cotyledons, roots, stems, leaves, immature bean pods, mature bean pods and mature beans. In all cases the tissues were analyzed for total metal radioisotopes.

In soil biochemical studies, enrichment techniques have been used to select for microorganisms that exhibit a high resistance to added metal concentrations. Procedures employing TLC, column chromatography, electrophoresis, and mass spectrometry are being developed to identify the metabolites of this resistance and/or detoxication process. These same analytical procedures are being used to characterize the chemical form of the metal in plant xylem, leaves, stems, and fruit.

In order to separate neutral organometallic complexes that had been made by the soil microbes, the total metal complex extract passed through a soil column. Charged complexes were adsorbed to humic and fulvic acid fragments of the soil, and neutral complexes which will most likely be transported to the root membrane passed through. The chemical characteristics of these neutral organometallic complexes were then determined by GS/MS.

MAJOR FINDINGS AND PROPOSED COURSE: From earlier studies, it had been demonstrated that soil microbes were capable of converting the inorganic metal salt into a series of organometallic complexes. In terms of mobility in soil solution and uptake by plants, however, the complexes of most importance are the electronically neutral ones. Using the soil perfusion column, neutral organometallic complexes of nickel produced by fungi have been isolated. Work is now in progress to determine the chemical characteristics of these complexes. The identification of the chemical constituents of the complex will indicate probable processes by which soil microbes modify metals, and the role of these processes in making metals more available for plant uptake. Similar studies are being carried out for cadmium, chromium, and thallium.

In kinetic studies with metal uptake by plants, studies are in progress to determine physiological processes that are important in transporting the metal across the root membrane and translocating it to different parts of the plant. These studies are continuing for nickel.

The biochemical studies which tie together the microbial studies and the uptake kinetic studies, are continuing to improve the selectivity, sensitivity, and efficiency of separation and identification procedures. Continued research is required to improve solvents, buffers, and chromatographic supports.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A number of avenues exist by which metals may enter the human food chain. The principal direct avenue probably results from using metals in agriculture as pesticides, desiccants or as contaminants of fertilizers. The importance of soil and plants in this pathway has been underscored by the spectre of metal conversion to more soluble and more toxic heavy metal complexes which may subsequently be taken up by plants and thereby enter directly into human foodstuffs. In related animal feeding studies with plutonium, it has been demonstrated that the rate of absorption from the GI tract for the organometallic complexes of plutonium in plants was much greater than for inorganic plutonium salts.

PUBLICATIONS

Cataldo, D. A., Garland, T. R., and Wildung, R. E.: Nickel in plants. I. Uptake kinetics using intact soybean seedlings. Plant Physiol. 62: 563-565, 1978.

Cataldo, D. A., Garland, T. R., and Wildung, R. E.: Nickel in plants. II. Distribution and chemical form in soybean plants. Plant Physiol. 62: 566-570, 1978.

Cataldo, D. A. and Wildung, R. E.: Soil and plant factors influencing the accumulation of heavy metals by plants. Environ. Hlth. Perspec. 27: 149-160, 1978.

MIDWEST RESEARCH INSTITUTE - Kansas City, Missouri 64110
(NIH-NIEHS-78-2153)

TITLE: An Appraisal of Environmental Exposure to Heavy Metals

CONTRACTOR'S PROJECT DIRECTOR: Ivan C. Smith, Ph.D.

PROJECT OFFICER (NIEHS): Warren T. Piver, Ph.D., Chemical Engineer,
Office of Health Hazard Assessment

DATE CONTRACT INITIATED: October 1, 1978

CURRENT ANNUAL LEVEL: $77,029

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this program are: (1) identify sources and routes of human exposure to heavy metals as a result of the various steps and industrial operations involved in the commercialization, utilization, and disposal of metals and their compounds; (2) examine and present available information on the toxicology of the metal and its compounds, and to identify analytical methods and their limitations for quantitative measurement of metals in environmental and biological media; and (3) forecast trends in metal production that may produce increased environmental entry and exposure to metals.

METHODS EMPLOYED: The methods used to perform these tasks are the use of literature reviews and expert opinions from industry in order to compile data on sources of release and exposure to metals and their compounds. Special interest is placed on determination of the physical-chemical properties of the metal that influence how the metal makes contact with man. The information is put together in a report on each metal of specific interest.

MAJOR FINDINGS AND PROPOSED COURSE: Within the past year, a report on indium has been produced. These reports have a special significance because they attempt to define the chemical state of the metal in the environment as a result of its release due to intended use or as a contaminant in air and water. Information is being gathered for a number of metals of importance from a toxicological viewpoint. A report on cobalt is in press. These reports have been published in book form and are available to a wide readership. After the report on cobalt is prepared, a report on the lanthanide series of elements is planned.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This surveillance is essential to stay abreast of changes in environmental contamination by certain industrial or communal practices involving different metals and their inorganic and organometallic derivatives and complexes. The information determines gaps in understanding on the health effects of the compound in question and the type of research to be undertaken. In addition, these documents have been sent to biomedical researchers who are with Federal and academic programs, and to program directors in foreign countries. Comments received from these people have indicated that these reports are timely and provide useful information for planning research programs.

PUBLICATIONS

Smith, I. C., Carson, B. L., and Hoffmeister, F.: Trace Metals in the Environment, Vol. 5 - Indium. Ann Arbor Science Publisher, Inc., Ann Arbor, Michigan, 1978.

INTERAGENCY PROGRAMS

OFFICE OF ASSOCIATE DIRECTOR FOR INTERAGENCY PROGRAMS
Summary Statement

The Associate Director for Interagency Programs is responsible for the following program areas:

US-USSR Cooperation

Collaboration between Soviet and American environmental health scientists is carried out under the auspices of two cooperative agreements between the United States and the Soviet Union. Under the *Medical Science and Public Health Cooperative Agreement*, scientists from both countries are conducting joint research on heart disease, cancer, arthritis, influenza and acute respiratory diseases, and health problems associated with environmental pollution. The Director, NIEHS, is U.S. Coordinator for the environmental health activities under the Health Agreement.

1979 was the seventh year of formal collaboration in environmental health research between the US and USSR. The first year was concerned largely with establishing working relationships and agreeing on areas of joint study. Cooperative research efforts were initiated in the second year of the agreement and involved exchange visits between scientists of both sides. The research results developed during the second and third years of collaboration were presented by American and Soviet scientists at a joint symposium in Riga, Latvia, in December 1974. Scientific results from cooperative research during 1975 and 1976 were presented at the second joint symposium, held in Marineland, Florida, in December, 1976. The results of these symposia were published in both countries. During 1978-1979, major workshops were held on the topics: "Biological Effects of Metals" (Cincinnati, February, 1978); "Behavioral Toxicology" (Suzdal, November, 1978); and "Biological Effects of Physical Factors in the Environment" (Seattle, June, 1979).

Collaborative research efforts are currently divided into three problem areas aimed at (1) development of approaches for the quantitative evaluation and prediction of the biological effects of environmental chemical agents; (2) study of the long-term biological effects of environmental chemical agents; and (3) study of the long-term biological effects of physical factors in the environment.

Over 50 scientific papers have been published by American and Soviet scientists on the results of environmental health research conducted to date under this agreement. In addition, a Russian-English Glossary of Environmental Health Terminology was published in both countries to assist the communications between scientists of both sides.

The *Agreement on Cooperation in the Field of Environmental Protection* between the US and USSR addresses some of the most significant aspects of problems in the environment and is focused on the area of biological and genetic effects of pollution. A number of agencies participate in this agreement, which is under the general direction of the Administrator, EPA. The Director, NIEHS, serves as HEW representative to the agreement and as Co-Chairman of the working group for the section on Biological and Genetic Effects of Pollution. Effort in this area has been focused on the mutagenic potential of environmental contaminants, the toxic effects of heavy metals in the environment, the toxicity of oil shale products and by-products, neuroendocrine effects, and the effects of pollution in the marine environment.

During 1978-1979, program activity under this agreement consisted of the fourth joint workshop on "Basic and Practical Approaches to Environmental Mutagenesis and Carcinogenesis" held in Baku, USSR, and participation in the joint workshop on the topic "Biological Effects of Metals", held in Cincinnati, Ohio. In addition, exchange visits were conducted in the areas of health effects of oil shale development, human behavioral effects, and marine organisms.

Energy-Related Research

As part of the high priority accorded by the Federal Government to solving the energy problems facing the nation, NIEHS is involved in a variety of activities to elucidate the potential adverse health impacts of energy technologies.

During the summer and fall of 1974, an OMB/CEQ task force met to identify needed research on the potential adverse health and environmental problems associated with energy use. As part of the report of the Interagency Working Group on Health and Environmental Effects of Energy Use published in November 1974, the Health Sub-Group, co-chaired by the Director, NIEHS, recommended that research be pursued in several areas. As a result of the work of this task force, NIEHS was assigned the responsibility, and given additional funds to develop research programs to address a number of important energy-related questions. The energy-related research being pursued by NIEHS focuses on mutagenic effects; teratogenic and reproductive effects; behavioral and neurotoxic effects; inhalation toxicity and pulmonary effects; subcellular, cellular and organ toxicity; pharmacologic effects and the determination of immediate and long-term effects of critical pollutants on selected ecosystems and organisms in the marine environment. During the period 1975-1979, NIEHS initiated a number of new projects focused on problems in these areas.

In January 1976, NIEHS co-sponsored, along with NIOSH, EPA, and ERDA, a retreat for 150 scientists working on energy-related health problems. In May 1977, the President directed HEW, EPA, and DOE to establish a joint program to identify the health and environmental effects of energy technology. The three agencies initiated a series of jointly sponsored scientific workshops for the purpose of obtaining an up-to-date identification of the health and environmental problems associated with each energy technology and the research needs required to address those problems. In April 1977, the President's Message on Energy announced the intention to appoint a special committee to study the health and environmental effects of increased coal production and use. The Director, NIEHS, was appointed Chairman of the Committee which reported its findings and recommendations to the President in December, 1977.

Interagency Coordination

During 1976-1977, a report on Federal Agency Support for Environmental Health Research was prepared at the request of the Senate Appropriations Committee. This report was updated in June, 1978 and May, 1979 at the request of the Director, Office of Science and Technology Policy, Executive Office of the President. The report summarizes the environmental health research responsibilities, functions, and coordination efforts of the Department of Health, Education, and Welfare, Department of Energy, Environmental Protection Agency, Department of Agriculture, Department of Commerce, Department of Interior, Department of Defense, National Science Foundation, Nuclear Regulatory Commission, National Aeronautics and Space Administration, Department of Housing and Urban Development, Department of Transportation, and Veterans Administration. These agencies have budgeted approximately $673 million for environmental health research in FY 1980.

As a service to the National Toxicology Program, the Office of Interagency Programs prepared a Review of Current DHEW Research Related to Toxicology. This review is required as a part of the annual plan for the National Toxicology Program. The first review surveyed agencies of the Public Health Service for information on their programs in basic toxicology research, toxicology testing, and toxicology method development. Seventeen PHS agencies reported funding for toxicology-related research amounting to approximately $201 million in FY 1978 and identified the chemical compounds being tested for a variety of biological endpoints.

PROGRAM PLANNING AND EVALUATION

OFFICE OF PROGRAM PLANNING AND EVALUATION
Summary Statement

During its second year of operation the Office of Program Planning and Evaluation (OPPE) continued to be drawn into an ever-widening circle of environmental concerns not narrowly defined as health related. This increased activity reflected three developments:

1. The growing public recognition that environment and health and other societal concerns are interrelated.
2. The increasing need for both the Congress and the public to understand environmental health issues which bear on problems which do not have health as their central focus.
3. Greatly increased Executive Branch activity in this area which requires that the Institute be able to focus on the interrelationships between the science, legislative, regulatory, and public policy components of environmental health issues.

The complexity and breadth of these interrelationships and issues effects the work of OPPE in a substantive way. In addition it requires that OPPE become increasingly "outward" oriented so that it can relate NIEHS programs to other research programs as well as related non-research programs and issues.

During FY 1979 OPPE carried out activities covering its entire range of responsibilities. These activities covered planning, evaluation and legislation. In addition a significant block of staff time was committed to providing assistance in the activities which led up to the establishment of the National Toxicology Program (NTP), developing operating procedures for the NTP Executive Committee, and developing a five-year plan for the NTP, as well as briefing papers on NTP. Throughout the year OPPE was called upon to prepare both analytical and descriptive papers covering a variety of subjects, and to review a variety of program documents, prepared by other agencies, covering a variety of science and public policy issues.

These activities are discussed below.

Program Planning Activities

OPPE continued its efforts to strengthen the planning function of the Institute. These efforts were aided by a number of factors:

1. The imminent completion of the permanent facility requires effective planning to take advantage of the research opportunities it will

provide. This was underscored by the requests of the House and Senate Appropriations Committee for a report detailing these plans.

2. The new Scientific Director embarked on an effort to develop a comprehensive and cohesive plan for the Intramural program.

3. The requirement for both an annual and five-year plan for the NTP required that the Institute develop plans for its contributions to the NTP.

The OPPE began the fiscal year with the preparation of the 1981-83 Research Plan. This process which includes extensive interaction with NIH planning staff and provides the opportunity for discussions between the Director, NIH and his senior staff, and the Director, NIEHS and his senior staff is particularly helpful to both OD-NIH and NIEHS. These discussions are particularly useful as briefing for the upcoming appropriations hearings. This interchange in many ways constitutes the most valuable part of this planning exercise. The utility of the formal part of the exercise is still open to some question in our view.

Program Evaluation and Analysis Activities

While OPPE has carried out a significant level of program evaluation activity, the press of ad hoc short deadline exercises and staff committment to NTP has precluded as much as would be desirable.

With regard to the formal set-aside evaluation activities the appointment of the new Deputy Assistant Secretary for Evaluation has had a salutary impact. There is not only greater responsiveness in the system but it has been opened to a level of informal interchange which is most useful. The Deputy Assistant Secretary has made real efforts to reach out to the agencies for their views and inputs. It would be pleasant to think that this openness has become institutionalized.

During the year, the Institute received approval for the use of set-aside funds for its evaluation of the NIEHS training program in environmental toxicology. The contract has been awarded for this study which is now in progress. It is hoped that this study will be a useful pilot for additional studies in the manpower area. Good data in this area is critical to developing programs responsive to national need.

Institutionally OD-NIH has begun to hold meetings between the Director, NIH and each of the Institute Directors to discuss the Institute's annual evaluation plans. These meetings are similar to those held to discuss the research plans, and in our view they are not as useful. One of the reasons is it is difficult to raise them to the appropriate level of generality. It is hoped that they will become more useful in the years ahead.

Legislative Analysis Activities

Throughout the year OPPE carried out a variety of legislative analysis and support activities. The issues which attracted the greatest attention were those surrounding asbestos. This included the preparation of bill analyses and testimony on asbestos in schools, and compensation issues growing out of occupational exposures to asbestos.

Issues which were attracting considerable attention at year's end and promise to become major environmental health issues in the coming year include compensation issues related to toxic chemical dumps, revision of occupational compensation programs to make possible more effective response to occupationally related chronic disease, radiation induced disease, the health effects of dioxin exposure, and the health effects related to increased use of existing energy sources, as well as new energy sources.

Related Professional Activities

The Director, OPPE participated in a panel at the Annual Meeting of the American Political Science Association which dealt with the work of the House Commission on Administrative Review.

Publications

"The National Institute of Environmental Health Sciences: A Perspective," by Elizabeth Y. James (now Hudson) and George M. Kingman, was published in the October, 1978 issue of Environmental Health Perspectives.

INTRAMURAL RESEARCH

OFFICE OF THE SCIENTIFIC DIRECTOR

OFFICE OF THE SCIENTIFIC DIRECTOR
Summary Statement

Dr. Charles E. Carter agreed to accept the position of Scientific Director, and assumed command during the second half of the year. Prior to coming to NIEHS, Dr. Carter had been serving in a dual capacity as Acting Director, Division of Health Effects Research, and Acting Director, Division of Health and Environmental Effects Assessments, Office of Health and Environmental Research, U.S. Department of Energy, Washington, D.C. Prior to Dr. Carter's arrival, Dr. David G. Hoel, Chief of the Biometry Branch, had served ably over an 18-month period as Acting Scientific Director.

Emphasis during the year was given to: (1) recruiting leaders for new programs in Animal Genetics, Molecular Genetics, and Organ Function and Toxicology; (2) coordinating and reviewing research plans and progress of the programs in the Institute's intramural research laboratories and establishing program priorities; (3) working with the Associate Director, Research Resources, the Deputy Director, the Chief, Research Services Branch and program leaders to finalize detailed plans for the permanent facility including physical location of programs, special requirements, support services, and animal needs; (4) working with the Associate Director, Research Resources, to relocate laboratory groups with minimal program disruption during renovation of Building 7; (5) working with the Director and Associate Director, Research Resources, and program leaders to define the role and commitment of intramural research to the National Toxicology Program; and (6) developing plans for review of programs by the Board of Scientific Counselors.

Accomplishments include the splitting of the Laboratory of Environmental Mutagenesis (LEM) and subsequent formation of two new laboratories, the Laboratory of Animal Genetics (LAG) and the Laboratory of Molecular Genetics (LMG), which encompass programs from the LEM. This reorganization should enable better administrative and scientific program management. Dr. John Drake, Acting Chief of the LEM, was named Chief of the LMG, and Dr. Burke Judd was recruited to serve as Chief of the LAG. The Laboratory of Organ Function and Toxicology (LOFT) was established from units split from the Laboratory of Environmental Toxicology for the purpose of providing a focus for toxicologic research on a number of important organs. Dr. George Lucier is Acting Chief, and a search committee was established and is currently evaluating prospective candidates for permanent Chief of the LOFT. An Institute Clinical Review Committee was established to evaluate and monitor proposed and ongoing research by Institute scientists involving human subjects and/or clinical samples. The Scientific Director served as an expert consultant in the organization and planning for the National Toxicology Program (NTP). Further, the Office of the Scientific Director coordinated and prepared the Institute's submission describing current research programs related to chemical toxicology for the first Annual Plan of the NTP. The office continues to provide coordination and input for intramural program participation in the Health and Ecological Effects of energy technologies pass-through program.

The Board of Scientific Counselors met twice during the year to review intramural research programs. Programs presented and reviewed by the Counselors included the Laboratory of Behavioral and Neurological Toxicology, the Laboratory of Environmental Biophysics, the Laboratory of Pulmonary Function and Toxicology, the Laboratory of Biochemical Genetics, and the Inhalation Toxicology Section of the Environmental Biology Branch. The Counselors will continue to review each of the intramural laboratories, branches and other major programs at least once every three years, and sometimes more often. The practice of supplementing the Board with ad hoc expert reviewers for each program will continue as it enables more in-depth review of program areas. Permission was received to expand the Board membership by two members, and new members were recruited representing the areas of mutagenesis and environmental chemistry.

A second NIEHS Science Seminar was coordinated by the Office of the Scientific Director, and held June 13-14, 1979, at the McKimmon Center, North Carolina State University, Raleigh, North Carolina. The purpose of the Seminar was two-fold: to provide in-depth information about Institute intramural research programs to scientists and administrators from NIH, other Federal agencies, industry and universities interested in or involved in environmental health sciences, and to give intramural staff an opportunity to learn about ongoing research in program areas other than their own. NIEHS scientists presented about 115 posters and several formal lectures and round table discussions during three half-day sessions. In addition to Institute staff, about 150 persons from outside the Institute participated. We propose to continue to have such sessions on a biennial basis.

The Scientific Director continues to work closely with the Intramural Research Council and its members (Laboratory and Branch Chiefs in the Intramural and Research Resources Programs) to solve various problems that affect the Intramural programs, such as personnel matters, training needs and allocation of resources and space.

The Scientific Director continues to meet regularly with other Scientific Directors at NIH in Bethesda to discuss various inter-institute matters such as appointment and promotion plans, policies and reviews, EEO and affirmative action plans, rules for recombinant DNA research, Board of Scientific Counselors reviews of intramural research, travel plans and policies, contracts and monitoring, employment ceilings and grades, central research and research support facilities (libraries, computers, clinical pathology), clinical research activities, post-doctoral and visiting scientist plans, policies and review, personnel management and supervisory training efforts. These meetings are at least twice a month and assure that institutes do not develop procedures and policies at wide variance or threatening to the concept and practices of NIH as a whole. There is also a sharing of information about the research programs at other institutes and this helped in collaboration, avoidance of unnecessary duplication, etc.

The Scientific Director's Office was called upon to present or organize programs describing the intramural research program to a variety of outside visitors and groups on a number of occasions. Among these were the NIEHS Center Directors, NIEHS Marine Center Directors, and the Chemical Industry R&D Directors.

Also, the office coordinated, provided information and/or prepared written reports on intramural research programs in response to requests from various governmental task forces or committees, such as the National Commission on Digestive Diseases.

BIOMETRY BRANCH

BIOMETRY BRANCH
Summary Statement

The Biometry Branch fulfills a dual role within the Institute's Intramural Research Program. Its primary emphasis is on the initiation of applied, environmental-health oriented research in the areas of biomathematics, risk assessment, and epidemiology. In addition the Branch has responsibility for providing statistical, mathematical, data processing and computer engineering support to the Institute.

Biomathematics research is concerned with the mathematical modelling for biological systems and involves collaboration with a variety of other research groups at the Institute. Within this research effort attention continues to be centered on the areas of mathematical population genetics and pharmacokinetic modelling. Risk assessment activities have emphasized improved utilization of data generated from high-dose-level animal screening studies to estimate long-term human risk associated with low environmental levels of exposure. Research is being conducted on a variety of issues that relate to different aspects of this general problem area. The Branch's epidemiology program attempts to assess the impact on human health of exposure to potentially hazardous compounds in the environment. Hazard assessments are made directly through actual field studies as well as indirectly by the analysis of pre-established data bases. Methodological research related to the improved planning and evaluation of epidemiological studies is also performed.

Statistical support provided to the intramural program covers a wide range of consulting activities from experimental design and data analysis to model estimation and simulation studies. An active research effort in the development of new analytic techniques is maintained in order to meet some of the highly specialized needs of various Institute scientists. Computer support covers such diverse activities as the creation of information retrieval and inventory maintenance systems, the simulation of complex biological models designed to gain insight into mechanistic processes, and the processing of large volumes of data in conjunction with statistical consulting activities. In addition, computer engineering support is provided for real-time data acquisition and minicomputer controls systems.

BIOMATHEMATICS

At the present time investigations are being conducted in three separate areas of mathematical biology. Two of these programs involve, to some degree, collaboration with other research groups within the intramural program. Joint research with the Laboratory of Animal Genetics is directed toward the derivation of more realistic models for certain phenomena in population genetics. As a result of this effort, a new model has been developed for estimating the nucleotide mutation rate through the use of restriction enzymes. Additional work is underway to compare this estimate with others that have been proposed in the literature. Attempts are being continued to adapt stochastic diffusion equation theory for the prediction of the effect of autocorrelated changes in the environment on gene frequency distributions.

Joint research with the Laboratory of Pharmacokinetics is directed towards the development of pharmacokinetic models and methods for quantifying the mechanisms of xenobiotic uptake, distribution, metabolism, and excretion in animals and man. Physiological compartmental models of the in vivo chemokinetics of several halogenated biphenyls have been constructed for the rat and efforts to scale these models to predict the fate of these compounds in other animal species are in progress. Collaboration with the Marine Pharmacology and Biomedicine Program in the Laboratory of Pharmacology has resulted in a preliminary model for the pharmacokinetics of selected organic pollutants in a marine animal, the spring lobster. This latter work is aimed at better understanding the potential accumulation of toxicants in human marine food sources.

Efforts are underway to adapt and improve existing models that are used to predict the number of mutants in growing bacterial populations. These models have become increasingly more important because of the current interest in the Ames test and other in vitro test systems that are used for detecting chemical mutagenesis. Currrent work is aimed at incorporating toxicity into the models and relating it to dose response curves.

RISK ASSESSMENT

The major focus of risk assessment research is on the development and/or modification of statistical procedures for estimating long-term human risk resulting from low-level exposure(s) to chemical carcinogens using data generated from animal carcinogenicity tests conducted at high, often maximum tolerated dose levels. A variety of research projects relating to different aspects of this problem are currently underway within the Branch.

The effect of introducing pharmacokinetic considerations into low dose extrapolation procedures is being investigated through computer simulation. Initial results suggest that the use of relatively general pharmacokinetic models may alter low dose risk estimates by an order of magnitude or even more.

The effort to quantify human cancer risk relative to various species of laboratory animals (for a series of carcinogens reviewed in the "Meselson Report") is being continued. In addition to considering the role of factors such as the site of action, relative species lifespan, and unit of dosage quantification, attention is also being directed toward biological factors that could potentially contribute to real species differences.

The question of model convergence at low doses is being investigated. As part of this effort, a measure of tail heaviness has been developed and is being applied to the standard extrapolation models.

Commonly employed methods for incorporating background rates into risk extrapolations have been assessed in depth. This evaluation process indicated that if even a very minor portion of the background is viewed as being additive, then the dose-response model will be essentially linear at low doses.

EPIDEMIOLOGY

Field studies of human morbidity due to environmental pollutants continue to play a major role in the Biometry Branch's epidemiology program; however, we also carried on basic and applied research in both statistical and laboratory support methodology. A prospective study, the Breast Milk and Formula project, is now in its second year. This study follows the growth, development, and morbidity of about 900 children from birth, and includes measures of organohalide contaminants (polychlorinated and brominated biphenyls, DDT and its metabolites) in the mothers' serum and breast milk. During this year, plans have been completed for extension of follow up from 6 months to 3 years.

A study of spontaneous abortion and the exposure of pregnant women to certain environmental mutagens and clastogens is now in the planning stages, and arrangements for the development of a valid and reliable survey instrument (questionnaire) have been made. This "disease" or pregnancy outcome is not only interesting in its own right, but also may serve as an indicator of serious and long lasting damage to the human genome, including neoplasia. Studies are also planned on the reproductive performance of workers exposed to radiation (plutonium) and a specific industrial chemical, perchlorethylene.

Another area of program activity is metal and trace element toxicity. The results of a pilot study on the relationship between blood pressure and blood lead levels in children were published this year. A new study on enzyme markers for susceptibility to the hematologic effects of lead has begun. A study of the role of selenium deficiency in the production of skin cancer is planned.

A new area for the program is the search for laboratory evidence of mutagenic exposures in human tissues. Such laboratory tests should prove to be powerful tools in field investigations of groups exposed to potentially harmful levels of mutagenic substances. Such laboratory evidence is also useful in investigation of the mechanisms by which environmental agents might produce genetic damage or neoplasia.

New work on the use of statistics in epidemiology has been published. We have examined the effects of exposure misclassification on power and bias in prospective studies, and the assessment of synergism associated with the joint action of two risk factors.

STATISTICAL CONSULTING

The Biometry Branch provides a comprehensive statistical consulting service for the intramural research program. This effort covers a wide range of activities in the areas of experimental design and data analysis.

In the area of experimental design one of the most frequent problems involves sample size determination. This is an important issue since it is essential before an experiment is undertaken to assess the feasibility of achieving the study objectives with the available resources. Consideration is also given to possible improvements in the basic design itself, so as to eliminate possible confounding factors and to insure optimum allocation of animals.

The Biometry Branch also provides data analysis support, including tabulation of summary statistics, curve fitting, significance testing, and interpretation of test results. These efforts are closely co-ordinated with the computing work group.

Frequently, applied statistical research results as a direct consequence of the Branch's consulting activities. For example, analysis of laboratory data from a recessive lethal study led to the development of a new statistical procedure for the detection of recessive lethal carriers. The role of genetic structure of test animal population on the statistical design of toxicity assays was studied. Various stopping rules for automated sequential animal weighing schemes were compared in an effort to find those which had best average sample size under normal conditions and were resistant to various abnormalities. Finally, investigation is continuing into possible mathematical models that can be used to extrapolate toxicological-teratological risk to low dose levels.

COMPUTING

The Biometry Branch has the responsibility of providing computing and data processing support to NIEHS. The activities accomplishing this support may be thought of as consisting of two cooperating and interlocking efforts, namely computer operations and programming, and computer engineering.

The computer operating and programming effort assumes the responsibility for maintaining NIEHS' PDP 11/70 computer system and a network of terminals connected to the various computers at NIH/DCRT, assisting the NIEHS community in its use of available computer systems, providing programming consultation services as required, providing software systems development capabilities to support intramural research efforts, and providing support and collaborative assistance to the computer engineering effort.

Of major importance to the data processing effort this FY has been the addition of eight contract analysts and programmers obtained through GSA/IDSF. This has allowed the development of software and systems which would not have been otherwise possible in view of our shortage of slotted positions. Two other activities which will ultimately lead to the existence of major systems involve the provision of consulting and systems analysis support to

1) the Comparative Medicine Branch for its microbiology and animal facility systems and 2) the Environmental Mutagen Test Development Program for its test compound information system. Additionally, information systems are being developed to serve the NIEHS library and to provide information management services to the National Toxicology Program. In direct support of the Biometry Branch's statistical data analysis activities, Penn State's "MINITAB" interactive statistical analysis package has been installed on the PDP 11/70. It is expected that improvements and extensions to this system will be undertaken during the next FY.

Development of the capability for providing computer engineering support to the laboratories of the Institute is also ongoing within the Biometry Branch. Solutions are being sought to engineering problems related to all aspects of computer hardware and software. Tasks within this effort have included the specification of minicomputers, peripherals, and vendor-supplied software; the design of timing devices and interfaces between minicomputers and laboratory instruments; and the development of software for control of experiments, data acquisition, data analysis, and data transfer. The following efforts are underway or were completed in the last year: (1) design support of computer interfaces; (2) computer system support of the development of a scanning microscope for biochemical genetics studies; (3) development of a multipurpose lab minicomputer adaptable to population genetics studies; (4) development of a minicomputer system for the acquisition of scintillation counter data in support of studies on prostaglandins and non-respiratory lung functions; (5) computer hardware support of the growth of the Biometry Branch central computer (PDP 11/70); (6) assisted in the design of a balance interface that links an electronic balance and standard terminal to a computer using a single serial line; and (7) planned a number of laboratory inventory software systems to be put on-line in the summer of 1979. In addition, a variety of computer support has been provided for the Laboratory of Behavioral and Neurological Toxicology. Most of this support has been software development including a variety of (1) data acquisition and experiment control, (2) manual data entry, (3) data reduction and reporting, (4) data base, (5) local data analysis, and (6) data communications software. General computer consulting support was also provided for a contract inhalation toxicology system, a joint NIEHS/NCHS data communications link to the Washington area, a future animal inventory and weighing system, and various terminal acquisitions.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 40002-09 BB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Statistical Methodology Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. K. Haseman	Mathematical Statistician	BB NIEHS
Other:	B. H. Margolin	Mathematical Statistician	BB NIEHS
	B. C. Gladen	Staff Fellow	BB NIEHS
	J. O. Koo	Visiting Fellow	BB NIEHS
	A. P. Davis	Visiting Associate	BB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was undertaken to develop new statistical methodology to deal with problems related to the Branch's consulting activities. There are a number of specific areas in which statistical research is being conducted. These include analysis of binary response data in certain toxicological experiments, mathematical modeling in the biological sciences, and power comparisons of various statistical procedures used in carcinogenicity testing.

PROJECT DESCRIPTION

METHODS EMPLOYED: Statistical techniques ranging from Monte-Carlo simulation procedures to analytical test development and mathematical modeling have been employed to address various statistical methodology problems arising from the Branch's intramural consulting activities.

MAJOR FINDINGS AND PROPOSED COURSE: (1) Monte Carlo studies are being conducted to compare the power of a number of statistical procedures proposed for detecting increases in lethal tumors. These include Cox's life table approach, Peto's test, and Breslow's procedure. In these simulations underlying Weibull distributions are being assumed to describe the survival of treated and control animals. (2) Various stopping rules for automated sequential animal weighing schemes were compared in an effort to find those which had best average sample size under normal conditions and were resistant to various abnormalities. Comparisons are not yet complete. (3) Models for binding of estrogen to receptors were studied in an attempt to discover models which fit various observed data sets. (4) A number of recently-proposed statistical procedures for the evaluation of binary response data in toxicological studies are being investigated. (5) The role of genetic structure of test animal population on the statistical design of toxicity assays was studied. If was found that if susceptible subgroups do exist and certain simplifying assumptions are made, then in many carcinogenicity studies tumor increases may be detected more readily by studying several inbred strains rather than a single outbred stock. (6) Investigation is continuing into possible mathematical models that can be used to extrapolate toxicological-teratological risk to low dose levels. The existence of litter effects increases the complexity of this problem.

Statistical methodological research will continue to form an important part of the overall support effort provided to the intramural research program.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The development of improved statistical methodology is essential if the Branch is to provide effective statistical consulting to the intramural research program.

PUBLICATIONS

Lin, F.A. and Haseman, J.K.: An evaluation of some nonparametric multiple comparison procedures by Monte-Carlo methods. Communications in Statistics, Part B; Simulation and Computation. B7(2): 117-128, 1978.

Schruben, L.W. and Margolin, B.H.: Pseudorandom number assignment in statistically designed simulation and distribution sampling experiments. Journal of the American Statistical Association. 73: 504-525, 1978.

Haseman, J.K. and Hoel, D.G.: Statistical design of toxicity assays: role of genetic structure of test animal population. Journal of Toxicology and Environmental Health. 5: 89-101, 1979.

Haseman, J.K. and Kupper, L.L.: Analysis of dichotomous response data from certain toxicological experiments. *Biometrics*. 35: 281-293, 1979.

Gladen, B.: The use of the jackknife to estimate proportions from toxicological data in the presence of litter effects. *Journal of the American Statistical Association*, in press.

Hamilton, M.A.: Robust estimates of the ED50. *Journal of the American Statistical Association*, in press.

Hamilton, M.A. and Haseman, J.K.: Statistical tests for recessive lethal carriers. *Mutation Research*, in press.

Hoel, D.G. and Gaylor, D.W.: Statistical analysis of carcinogenesis data from chronic animal studies. In Sontag, J.M. (Ed.): *Carcinogens in Industry and Environment*, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 40004-02 BB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Statistical Methods in Epidemiology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. C. Gladen	Staff Fellow	BB NIEHS
Other:	W. J. Rogan	Medical Officer	BB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to conduct research on statistical methodology problems related to the Branch's activities in the field of epidemiology. The objectives are both to broaden understanding of the uses and limitations of currently employed study designs and corresponding analyses; and to develop new techniques for statistical analyses of epidemiological studies.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Statistical techniques for the analysis of various kinds of epidemiological studies were devised or evaluated. Both theoretical mathematical calculations and computer simulations were used to assess techniques.

MAJOR FINDINGS AND PROPOSED COURSE: (1) Investigations of various formulations of interaction in a case-control framework were undertaken. For each one, the corresponding formulation in a cohort framework was shown. (2) Preliminary investigations of the effect of stratified sampling in a case-control framework were undertaken. Effects on such things as selection bias and efficiency were studied.

The proposed course is to continue research efforts on statistical methodology problems that arise in connection with the epidemiological investigations carried out by the Biometry Branch.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The understanding and criticism of current statistical techniques and the development of improved techniques is important for the proper evaluation of the results of epidemiological studies.

PUBLICATIONS

Hogan, M.D., Kupper, L.L., Most, B.M., and Haseman, J.K.: Alternatives to Rothman's approach for assessing synergism (or antagonism) in cohort studies. American Journal of Epidemiology. 108: 60-67, 1978.

Kupper, L.L. and Hogan, M.D.: Interaction in epidemiologic studies. American Journal of Epidemiology. 108: 447-453, 1978.

Gladen, B. and Rogan, W.J.: Misclassification and the design of environmental studies. American Journal of Epidemiology. 109(5): 607-616, 1979.

Hamilton, M.A.: Choosing the parameter for a 2x2 table or a 2x2x2 table analysis. American Journal of Epidemiology. 109: 362-375, 1979.

Rogan, W.J. and Brown, S.M.: Some fundamental aspects of epidemiology: a guide for laboratory scientists. Federation Proceedings. 38: 1875-1879, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 40005-02 BB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Statistical Analysis of Mutagenesis Testing Data

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. H. Margolin	Mathematical Statistician	BB NIEHS
Other:	N. Kaplan	Research Mathematician	BB NIEHS
	B. Collings	Mathematical Statistician	BB NIEHS
	E. Zeiger	Acting Head, EMTDP	LMG NIEHS

COOPERATING UNITS (if any)

Laboratory of Molecular Genetics

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.3	PROFESSIONAL: 1.3	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long-term objective of this project is to develop appropriate statistical techniques for the analysis of data arising from the Mutagenesis Testing Program. Focus has been on data from microbial test systems. Statistical procedures currently employed by other researchers in mutagenesis for the design and analysis of microbial experiments have been studied, and new and improved procedures have been devised.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Two microbial experiments for mutagenicity, the fluctuation test of Green, Muriel and Bridges, and the Ames test, were studied from a statistical standpoint. Modeling of the fluctuation test led to important conclusions concerning the design and analysis of this type of experiment. Continued stochastic modeling of the Ames test was conducted to permit toxicity and interlaboratory variability. Stochastic growth models that incorporate competing risks of mutation and toxicity are the new focus in this effort.

MAJOR FINDINGS AND PROPOSED COURSE: (1) Published recommendations for the design of fluctuation experiments are too loose in their specification of acceptable control rates of positive tubes and numbers of replicates. (2) The method of analysis proposed by the originators of the fluctuation test can be substantially improved for both single dose and multi-dose experiments. (3) The conclusions drawn from a fluctuation test can be insulated against a small proportion of aberrant observations. (4) New stochastic models constructed for the Ames Test data permit the inclusion of a random effect component; this will aid in the analysis of data from a variety of laboratory sources.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This project has altered the ways in which certain microbial test data are analyzed. The new methods of design and analysis will reduce the percentage of false declarations of positive or negative findings resulting from use of these microbial tests. This research effort is yielding methodological results of interest to biometrists in numerous other areas of research.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 41001-05 BB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Risk Assessment for Environmental Carcinogens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. G. Hoel	Chief	BB NIEHS
Other:	A. P. Davis	Visiting Associate	BB NIEHS
	M. D. Hogan	Mathematical Statistician	BB NIEHS
	N. Kaplan	Research Mathematician	BB NIEHS
	M. Anderson	Mathematician	LPK NIEHS
	T. Yanagimoto	Visiting Scientist	BB NIEHS

COOPERATING UNITS (if any)

Louisiana Tech University, Department of Mathematics

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The overall purpose of this project is to develop improved statistical techniques for using data from animal carcinogenicity tests conducted at high dose levels to estimate long-term human risk from chemical carcinogens at very low dose levels. Current research efforts have focused on such topics as the evaluation of competing risks and pharmacokinetics in the framework of multistage models for carcinogenesis as well as the question of species-to-species extrapolation. Also the question of model convergence at low doses and the incorporation of background is studied.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: (1) The effect of pharmacokinetic considerations on low dose estimation is being studied via computer simulation techniques. (2) Meselsons's NAS estimates of human relative cancer risk--where relativity is expressed in terms of the most sensitive animal species tested--for a variety of known carcinogens are continuing to be re-examined in order to determine the extent to which they depend on both the length of exposure and the unit of dosage quantification. (3) The structure of the tails of dose response distribution are being theoretically studied. (4) A generalized model for background incorporation is being developed.

MAJOR FINDINGS AND PROPOSED COURSE: (1) For fairly general pharmacokinetic models estimates at low doses may change by an order of magnitude. The simulation studies will continue. (2) It was found that when relative lifespan and body surface area were introduced into the animal versus man risk calculations for the carcinogens considered by Meselson, the similarity between species was even stronger than he originally indicated. (3) A measure of tail heaviness was developed and is being applied to the common extrapolation models. (4) It has been shown that if only a small portion of background is incorporated in an additive manner then the dose response model is essentially linear at low doses. This has the effect of bringing together the many different models which have been under study for risk estimation purposes.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: As animal experimentation plays an ever-increasing role in the assessment of human cancer risk, the importance of improved statistical procedures for realistically assessing this risk is obvious.

PUBLICATIONS

Hoel, D.G.: Some problems in low dose extrapolation. In Hiatt, H.H., Watson, J.D., and Winsten, J.A. (Eds.): Origins of Human Cancer. Cold Spring Harbor Laboratory, 1977, Vol. 4, Book C, pp. 1391-1396.

Hoel, D.G: Low-dose and species-to-species extrapolation for chemically induced carcinogenesis. In: Banbury Report I "Assessing Chemical Mutagens: The Risk to Humans." Cold Spring Harbor Laboratory, 1979, pp. 135-145.

Hoel, D.G.: Animal experimentation and its relevance to man. Proceedings from US-Japan Conference on Biostatistics in the Study of Human Cancer, May 1978. Environmental Health Perspectives, in press.

Hoel, D.G.: Sequential methods in genetic risk assessment. Genetics 92, 1979, in press.

Schoenfelder, C.A. and Hoel, D.G.: Properties of the Neyman-Scott carcinogenesis model at low dose rates. Mathematical Biosciences, in press.

Hoel, D.G.: Statistical approaches to toxicological data. Proceedings, 5th Symposium on Statistics and the Environment, NAS. Environmental Health Perspectives, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 42002-07 BB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Pharmacokinetic Modeling and Methodology Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. B. Tuey	Staff Fellow	BB NIEHS
	H. B. Matthews	Research Chemist	LPK NIEHS
	M. Anderson	Mathematician	LPK NIEHS
Other:	N. M. Morales	Visiting Fellow	LPK NIEHS
	R. L. Dedrick	Section Head	BEIB DRS
	R. J. Lutz	Chemical Engineer	BEIB DRS

COOPERATING UNITS (if any)

Chemical Engineering Section, BEIB, DRS, NIH
Laboratory of Pharmacokinetics

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to develop pharmacokinetic models and methods for quantitating the mechanisms of the uptake, distribution, metabolism, and excretion kinetics of environmental xenobiotics in various animal species. Mathematical models of the in vivo pharmacokinetics of several halogenated biphenyls in the rat have been developed and have been scaled to the mouse. Other models have been constructed to describe the disposition of these environmental contaminants in animals following chronic exposure. The pharmacokinetics of xenobiotics in marine animals that serve as human food sources is also being modeled. These mathematical models are intended to provide functional strategies for helping to extrapolate the disposition of chemical contaminants in the environment from one compound to another, and from one animal species to another, ultimately to include man. The evaluation and implementation of existing techniques and the development of new methodologies needed to accomplish these objectives are an integral part of this project.

PROJECT DESCRIPTION

METHODS EMPLOYED: The physiologic modeling approach used in drug disposition is being employed to develop similar type models for environmental contaminants. Computational algorithms for solving the equations that describe these models are being implemented.

MAJOR FINDINGS AND PROPOSED COURSE: (1) Physiological compartmental models have been constructed to describe the individual disposition kinetics of several different polychlorinated biphenyl (PCB) compounds in the rat. The tissue-level time-courses predicted by the models were generally consistent with the experimental data. Intestinal reabsorption was significant for metabolites of the lower chlorinated compounds. Animal growth was found to have a significant dilution and redistribution effect on the tissue levels of the slowly metabolized, highly lipophilic 2,4,5,2',4',5'-hexachlorobiphenyl compound. Skin tissue may not be perfusion-limited as assumed in the present model. Metabolism is the rate-determining step for the elimination of these compounds. The pharmacokinetics of 3,5,3',5'-tetrachlorobiphenyl showed that the positions of biphenyl chlorination can have more effect on the rate of metabolism than the degree of chlorination. (2) When allometric equations were used to scale these models to predict disposition kinetics in the mouse, good results were obtained for the more persistent, slowly metabolized isomers. (3) Whole body elimination kinetics after a single i.v. dose to mice was used to predict the body burden in other mice that received multiple oral doses of the same PCB compound. (4) A physiological compartmental model constructed from data obtained from rats that received a single i.v. dose of 2,4,5,2',4',5'-hexabromobiphenyl was able to simulate and predict the tissue levels found in rats placed on a 30-day chronic oral dose schedule. (5) A blood flow-limited physiological pharmacokinetic model was used to describe the overall time-course of octane and hexadecane disposition kinetics in the lobster, which possesses an open circulatory system. This research revealed that the crustacean shell is an important storage depot for these compounds. (6) A variable order, variable step-size numerical integration algorithm was found to be both computationally stable and several orders of magnitude more efficient than previously used predictor-modifier-corrector methods for solving multiple-dose pharmacokinetic model equations.

Future work will include attempts to refine the PCB models and account for small discrepancies which may prove significant in long-term studies, and to scale these models to the dog and the monkey. Compound-to-compound extrapolation of long-term PCB disposition kinetics based on short-term excretion kinetics and chlorination pattern will also be investigated. Mathematical models will be developed for the pharmacokinetics of additional aquatic contaminants and in other marine animals, and further refinements will be made to the general purpose numerical integration algorithm now available on this Institute's computer system.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An ability to quantitate pharmacokinetic similarities and differences from species to species and from compound to compound will enable better judgements to be made of the relevance of animal study results to man.

PUBLICATIONS

Morales, N.M., Tuey, D.B., Colburn, W.A., and Matthews, H.B.: Pharmacokinetics of multiple oral doses of selected polychlorinated biphenyls in mice. Toxicology and Applied Pharmacology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 43001-07 BB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Demographic Investigations of Potential Human Health Hazards

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. D. Hogan	Mathematical Statistician	BB NIEHS
	D. G. Hoel	Chief	BB NIEHS
Other:	S. Cordier	Visiting Associate	BB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.8	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The overall objective of this project is to identify and/or confirm the presence of various potential health hazards in the general environment through the mechanism of demographic investigations. Current research activities are concerned with the development of quantitative estimates of both the extent of potential occupational exposure to asbestos since the World War II era and of its impact on current and future overall cancer mortality in the United States.

PROJECT DESCRIPTION

METHODS EMPLOYED: Results from various wartime and post war surveys were used to develop estimates of the number of workers potentially exposed to asbestos in (1) the World War II shipyards, and (2) all other occupational categories. These projections were combined with literature based estimates of cancer risk to approximate the number of excess cancer deaths anticipated in the future among the survivors of this asbestos-exposed cohort.

MAJOR FINDINGS AND PROPOSED COURSE: (1) At least 7-8 million workers have been (potentially) exposed to asbestos since the onset of World War II, and over half are still alive and at risk of developing asbestos-associated cancer. (2) The maximum effect of this occupational asbestos exposure should be seen sometime in the next decade. However, excess cancer deaths should continue to be observed into the next century. (3) Excess cancer deaths associated with asbestos exposure will (at least at their peak) account for as large a percentage of the total cancer death toll as is sometimes attributed to all occupational exposures.

A project to develop computerized mapping techniques for investigating geographic variability in various pregnancy outcome indices was terminated when the results from a North Carolina based pilot study indicated that the data at hand was not sufficiently sensitive to justify expansion of the program.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The results of this research provide some indication of the extent to which the role of occupational exposures in overall cancer incidence and mortality has been under estimated.

PUBLICATIONS

Hogan, M.D., Chi, P-Y, Mitchell, T.J., and Hoel, D.G.: Association between chloroform levels in finished drinking water supplies and various site-specific cancer mortality rates. Journal of Environmental Pathology and Toxicology. 2: 873-887, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 43002-03 BB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Environmental Contaminants of Infant Foodstuffs - The Breast Milk and Formula Project

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. J. Rogan	Medical Officer	BB NIEHS
Other:	B. C. Gladen	Staff Fellow	BB NIEHS
	J. D. McKinney	Supervisory Chemist	EBCB NIEHS
	D. B. Walters	Chemist	EBCB NIEHS
	A. Bagniewski	Mathematical Statistician	BB NIEHS

COOPERATING UNITS (if any)

Environmental Biology and Chemistry Branch

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	1.3	0.4

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Polychlorinated biphenyl (PCB) contamination of breast milk in the ppb range is well documented, but health effects on infants fed such milk are unstudied. This project involves: (1) establishment of a cohort of breast and formula fed infants; (2) development of methodology to obtain reliable and reproducible samples of human body fluids and tissue from mother and child; (3) development of reliable methods for analysis of PCB's and other related parameters; (4) development and application of statistical procedures for the analysis of data generated from the study; and (5) evaluation of the children for specific outcomes thought to be related to organochlorine exposure due to PCB's.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The study is a prospective, or follow-up study. Field personnel have been hired, trained in protocol administration, and work at selected hospitals. Subjects are enrolled, informed consent is obtained, and a questionnaire administered to each mother at approximately the time of delivery. Samples of milk, formula, colostrum, placenta, and maternal blood are collected.

These samples are subjected to gas chromatographic and neutron activation analysis for PCB's, DDE(1,1-bis(p-chlorobiphenyl)-2,2-dichloroethane), total organic chlorine (TOCl) and bromine (TOBr), and total soluble organic chlorine (TSOCl) and bromine (TSOBr) in the ppb range.

The children are examined, and follow-up appointments made. Serial examinations are performed over the first 6 months of the child's life including 2 behavioral evaluations, one at birth and one at 6 months.

MAJOR FINDINGS AND PROPOSED COURSE: No data analysis is yet complete. Data collection in North Carolina will continue. Major progress to date has been enrollment of about 1/4 of participants and the perfection of techniques required for collection of samples without possibility of contamination. Quality assurance (QA) procedures for control of chemical analyses and the development of methodology for reliable and reproducible ppb level determination of PCB, DDE, TOCl and TOBr have been completed for milk and blood. Similar QA work is continuing for formula and placenta (TSOBr and TSOCl only). Analysis of complete sets of mothers' samples is being initiated.

The remaining objectives are: (1) to establish the relationship between milk levels, maternal blood levels, cord blood levels, placental levels, and colostrum at birth, and examine the trends in milk concentration over time; (2) to investigate the relationship between PCB and TOCl levels in the neonate and a number of specific outcomes; (3) to follow breast-fed and non breast-fed infants over a period of six months and look for differences in incidence of a number of specific outcomes; (4) to generate other hypotheses about toxic effects of chronic low dose PCB exposure in children; and (5) to establish a cohort of children for follow-up studies.

Plans for long term evaluation of these children are also being made.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The health effects of these low dose environmental pollutants are not well studied in children, and this project should allow identification and quantification of those that occur short term in this group. The methodology for studying such phenomenon is also of interest, and the development of a field efficient method for study of low level pollutants, such as PCB's, in humans is important.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 43003-01 BB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Studies in Pediatric Lead Exposure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. J. Rogan	Medical Officer	BB NIEHS
Other:	B. C. Gladen	Staff Fellow	BB NIEHS
	C. H. Langley	Research Geneticist	LAG NIEHS

COOPERATING UNITS (if any)

Laboratory of Animal Genetics

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Lead exposure and undue absorption occurs not only in inner city children exposed to paint but also in children around smelters and those whose parents work with lead. Although the encephalopathy and renal disease of acute lead intoxication are well described, other toxicities of lead as well as factors that alter susceptibility need further research. We have completed a pilot study on lead levels and blood pressure in children, and have begun investigation into genetic differences in susceptibility.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The study of lead and blood pressure was a record review of children screened in the New Hanover County. North Carolina Health Department Forms were developed in collaboration with local personnel; data analysis was done in-house by Branch personnel. Investigation into genetic variability in susceptibility to lead began with an attempt to separate isoenzymes of amino levulenic acid dehydrase, one of the enzymes inhibited by lead. This work was done at the Medical University of South Carolina. Both human and mouse ALA-D was used. Methods were developed for this enzyme using isoelectric focusing and polyacrylamide gel electrophoresis.

MAJOR FINDINGS AND PROPOSED COURSE: The isoenzyme study showed that ALA-D does have isozymes, and that they probably differ in their inhibition by lead. However, the methodology is not sensitive enough for clinical use in humans. Dr. Allen at MUSC is planning to pursue this independently.

We are now trying to identify a group of children who show above or below average porphyrin elevation to a given level of lead, and characterize them in terms of amount and inhibitability of ALA-D. Since the amount of the enzyme is not known to be inducible, finding such a difference would be evidence for genetic variation in susceptibility to the effects of lead on blood.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Lead is an ubiquitous pollutant, and children are most vulnerable to it. Lead absorption can be both prevented and treated. Thus, an understanding of its human toxicity is appropriately within the NIEHS mission. Current screening programs continue to find and have treated children with undue lead absorption. The decision to treat is made on the basis of laboratory tests rather than clinical illness, and thus it is likely that some children are treated unnecessarily. Successful identification of children with greater susceptibility would allow more effective screening for children likely to show toxicity.

PUBLICATIONS

Rogan, W.J., Hogan, M.D., and Chi, P-Y.: Blood pressure and lead levels in children. Journal of Environmental Pathology and Toxicology: 2: 517-519, 1978.

Barr, M., Jr., Keller, C.A., and Rogan, W.J.: A summary of the workshop on perinatal and postnatal defects and neurologic abnormalities from chemical exposures. Annals New York Academy of Sciences, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 43004-01 BB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Skin Cancer and Trace Elements

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Clark	IPA	BB NIEHS
	S. M. Brown	Medical Officer (Research)	BB NIEHS

COOPERATING UNITS (if any)

To Be Determined

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this work is two-fold: first, to examine the hypothesis that there is a relationship between the etiology of skin cancer and the levels of certain trace elements in blood or hair. The elements of interest include selenium, arsenic, cadmium, zinc, copper, and lead. The issue relating to selenium is of the greatest interest. Second, the hypothesis that occupational exposure to herbicides, pesticides, or fungicides, including those containing arsenic as well as other groups of aromatic or aliphatic hydrocarbons, is a significant risk factor for the development of skin cancer. A case-control design in which subjects from a specific dermatologic clinic practice in the eastern part of North Carolina will be selected is anticipated. Four groups of skin cancer patients of different types will be compared with a group of dermatologic controls (persons with skin diseases other than, and unrelated to, skin cancer). The disease outcome of interest is skin cancer, and the risk factors of interest are selenium level, other trace element levels, and occupational exposure.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The current year has been occupied with the planning of this study, including investigation of its feasibility and laying plans for field work upon which the study rests. Contact with an appropriate practice, in a low selenium area, has been made where more than 900 patients are available for study. A computer printout of the demographic and histopathologic distribution of these patients is available. Sample size calculations have been performed in order to ascertain the number of patients in each study group required to be able to detect a decrease of twenty percent below the mean expected selenium level. This calculation assumes a power of ninety percent and a significance probability of less than five percent. The four groups of study patients include persons whose skin cancers are thought to have an ultraviolet origin, persons whose tumors are thought not to have an ultraviolet radiation origin, persons whose tumor at incidence was an isolated event, and persons whose tumor at onset was part of a multiplex event (defined either as simultaneous or sequential multiple skin tumors, thought to be separate incidences). The laboratory tests for trace element levels will be performed as a service contract through a laboratory able to process such samples. A number of other laboratory tests are planned to provide information concerning the nutritional status of the patients in controls, since such status is known to affect trace element levels. Etiologic hypotheses relating to arsenic (known to be of interest historically in the skin cancer literature), cadmium (and cadmium/zinc ratio), and certain other trace elements will also be investigated. Sample size calculations make it likely that the power of the test for selenium will be approximately of the same magnitude for most, but not all, of the other trace element levels. A multivariate analysis is planned in which covariables known or suspected to be of interest are taken into account. Interview of the patients by the treating physician and/or his/her staff will be the method employed to ascertain occupational or exposure history studied. The latter information considered of secondary importance in this study but will be pursued because of its accessibility.

MAJOR FINDINGS AND PROPOSED COURSE: No findings are currently available. The literature involving the etiology and pathogenesis of skin carcinogenesis has been thoroughly reviewed. In addition, the literature relating to hypothesized mechanisms of selenium carcinogenesis and selenium protection in the carcinogenic process have all been reviewed. The proposed course of study would be a six-twelve month period.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Skin cancer patients provide a unique opportunity for the investigation of etiologic factors for a number of reasons. First, and foremost, they are alive and "well", and for every skin cancer incidence, there is usually an available patient for study and in fact for subsequent restudy. In addition, the investigation of the general hypothesis that trace elements are related to the etiology of human cancer has been under-investigated. There is a low selenium geographic area in the state of North Carolina providing a unique opportunity for this investigation. Coincidentally, there is a large clinic-based practice in which skin cancer patients have been and are being followed for a relatively long period by an interested clinician. This set of coincidental facts provide the framework for a series of related investigations about the etiology of a form of cancer which has been largely presumed to be due to ultraviolet radiation. However, the issues of synergism in the etiology of these tumors, as well as the questions about that significant minority of patients whose etiology is probably not caused by exposure to sunlight, remain pertinent facts to be investigated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 43005-01 BB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Reproductive Experience of Drycleaning Workers and Their Conjugal/Marital Partners

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Wilcox	IPA	BB NIEHS
Other:	S. M. Brown	Medical Officer (Research)	BB NIEHS
	A. Blair	Staff Fellow	EEB NCI
	R. Hoover	Associate Chief	EEB NCI

COOPERATING UNITS (if any)

Environmental Epidemiology Branch, National Cancer Institute

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.4	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective is to complete a feasibility study on the possibility of contacting a suitable number of female drycleaning workers who are members of a labor union in St. Louis, Missouri. This union has been under study for cancer mortality by the Environmental Epidemiology Branch at the National Cancer Institute. The effort of the feasibility study is to determine whether or not a sufficient sample of female workers is available and willing to cooperate in an interview survey of their reproductive experience. The major study of interest would focus on the current rate of spontaneous abortion and other reproductive outcomes including birth defects. A second issue under consideration is whether or not tissue samples of parents of fetuses should be studied for cytogenetic damage.

PROJECT DESCRIPTION

METHODS EMPLOYED: This study is still in the planning stage. At the present time, if the feasibility portion of the study leads to the conclusion that a sufficient sample size is available, which is likely, a cohort study methodology is envisioned in which the comparison group would be members of a corresponding labor union such as the laundry workers in the same city. The feasibility study has two phases. In Phase One, which is almost complete, documentation of the numbers and demographic distribution of the union membership is made according to their cohort (period worked). An attempt will be made to access the number of persons who can be traced and are willing to be interviewed, by age and cohort. A sample of these will be asked for their fertility experience with respect to time, in order to access not only the number of women available, but the number of births available during three relevant periods: (1) before exposure; (2) during exposure (employment period); and (3) after exposure. Since number of births is the denominator on which spontaneous abortion rate is computed and similarly on which birth defect rate is computed, the adequacy of the sample size depends upon the number of relevant pregnancies and/or births. If this size requirement is satisfied, which is likely, then the protocol will be finalized for an actual survey of the outcomes mentioned using direct interview. It is likely that that survey will include blood samples of employees for evidence of cytogenetic damage. If these findings are positive, a second phase would focus on cytogenetic damage of relevant offspring.

MAJOR FINDINGS AND PROPOSED COURSE: It is estimated that 485 live births are required in each group (study, comparison) in order to detect a relative risk of 1.8 with a power of ninety-five percent at the five percent probability level; a sample of 393 would be required if the power were ninety percent. The latter is the required sample size. This sample would be of relevant pregnancies, i.e., pregnancies in the study group during a relevant period related to exposure to the solvent perchlorethylene used in drycleaning establishments. Consultation with persons interested in mutagenic chemical activity have been consulted in planning this project. The proposed course of study would be approximately 36 months, following the feasibility study, commencing approximately March 1, 1980.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The response of man to environmental mutagens, particularly via fetal wastage or damage, is an important health response in itself and also as an indicator of other more serious and long range damage to the genome. A cohort methodology would permit the assessment of exposure to one known mutagenic substance which may well be a human carcinogen, since it has been demonstrated to be a carcinogen in experimental mammals. This methodology will serve to assess rather precisely the relative risk of the health endpoints mentioned above, with respect to exposures whose actual dosimetry can be approximately stated. The implications of the outcome of this study relate to both genetic damage and neoplasia.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 43006-01 BB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Genetic Effects, Reproductive Outcomes, and Birth Defects and Occupational Exposure to Plutonium

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. M. Brown	Medical Officer (Research)	BB NIEHS
	D. Sandler	Epidemiologist	BB NIEHS
Other:	D. G. Hoel	Chief	BB NIEHS

COOPERATING UNITS (if any)
Los Alamos Scientific Laboratory, University of California
Department of Epidemiology, University of Minnesota School of Public Health

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.3	PROFESSIONAL: 1.1	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The principle objective is to examine the reproductive outcomes principally of spontaneous abortion but also congenital defects and perhaps childhood cancer among a cohort of females associated with five federal facilities in which there was significant occupational exposure to plutonium. The work will be performed in two stages. In the first stage, a cohort study of female workers at the five facilities is entertained. The protocol of that study is currently being planned. The second stage would study the same outcomes in the wives or conjugal partners of exposed male workers at these same facilities. An attempt will be made to ascertain the existence of a dose-response relationship, at least for the most common outcome (spontaneous abortion).

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: This study is currently in the active planning phase. It will be a collaborative effort between Biometry Branch, NIEHS, and the Health Study of Workers Group of the Los Alamos Scientific Laboratory. Through a contract with the University of Minnesota, a predoctoral candidate in epidemiology and environmental health and clerical personnel from Los Alamos Scientific Laboratory will perform a detailed investigation of the personnel and health records for several thousand workers in the groups described in the Summary of Work. According to a protocol designed by several members of the Biometry Branch, in collaboration with the Head Epidemiologist at LASL, a protocol will be developed in steps so that a stage one protocol will have been completed by June 30, 1979. NIEHS will have a principal role not only in study design and administration of the protocol, but in data analysis and preparation of publications, presentations, and testimony if any be required. Consideration is currently being given to the appropriate follow-up methodology for locating living offspring of exposed workers, in order to ascertain their medical status and history. Also under consideration are which, if any, laboratory tests should be utilized in executing a study of this magnitude. Such details have not yet been resolved.

MAJOR FINDINGS AND PROPOSED COURSE: No findings are currently available from this study. The literature involving spontaneous abortion and plutonium has been, and is currently being reviewed. Numerous preparatory activities involving interagency cooperation, study methodology, and funding have already been undertaken, and formal meetings scheduled. Proposed course of stage one of this study would be 24-36 months, and of stage two, 24-36 months. Direct surveillance of individuals by telephone interview, and of medical or hospital records of offspring, as well as vital records including birth and death certificates, is anticipated for both stages of the study.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The reproductive response of human beings to radiation exposure, particularly via fetal wastage and teratogenesis, is a poorly understood area. It is potentially a peculiarly fruitful one because of the frequent existence of relatively reliable dose information in a retrospective situation permitting cohort analysis and hence relatively precise estimation of risk. Furthermore, because of the growth of the peace time and defense uses of atomic energy in general, and the likely continued use of an exposure to radioactive materials including plutonium and other transuranic elements, it is of importance to estimate dose-response relationships involved in genetic effects or genetic damage to human beings resulting from exposure to these substances.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 44002-04 BB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Mathematical Population Genetics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. L. Kaplan	Research Mathematician	BB NIEHS
Other:	C. H. Langley	Research Geneticist	LAG NIEHS

COOPERATING UNITS (if any)

Laboratory of Animal Genetics

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this work is to construct and analyze stochastic models for phenomenon arising in population genetics. This work is being done in collaboration with scientists in the Laboratory of Animal Genetics. Current areas of interest are (1) extensions of a model previously developed to estimate the nucleotide mutation rate from restriction enzyme data and (2) continued development of areas of stochastic differential equations which are appropriate to population genetics and other biological areas.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Collaborative research was conducted on problems in population genetics with scientists in the LAG. This research, which augments the population genetics studies performed by the LAG, was probabilistic in nature, emphasizing Markov chain theory and diffusion techniques.

MAJOR FINDINGS AND PROPOSED COURSE: (1) A model has been developed to estimate the nucleotide mutation rate using restriction enzyme data. Computer simulations are being carried out to study the behavior of the estimates. Various extensions of the model are also under consideration. It is anticipated that this work will be completed by the end of this year. (2) Work is currently underway to weaken the classical independence assumptions that are normally made for certain diffusion approximations that are made in population genetics. Randomizing the environment is of particular interest. Attempts are also underway to prove strong convergence theorems for some of these diffusions.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: More realistic models for genetic phenomenon should be beneficial in predicting long-term effects of environmental changes on public health.

PUBLICATIONS

Gillespie, J.H. and Guess, H.A.: The effect of autocorrelation on the progress of natural selection in random environments. American Naturalist. 112(987): 897-909, 1978.

Kaplan, N. and Langley, C.: A new estimate of sequence divergence of mitochondrial DNA using restriction endonuclease mappings. Journal of Molecular Evolution, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 44003-02 BB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Fetal Wastage and Chemical Exposure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. M. Brown	Medical Officer (Research)	BB NIEHS
Other:	A. K. Bagniewski	Mathematical Statistician	BB NIEHS
	D. Sandler	Epidemiologist	BB NIEHS

COOPERATING UNITS (if any)

To be Determined by Request for Proposal (RFP)

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.8	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective is to examine the variations in response of pregnant women who when exposed to any of a number of defined environmental mutagens and clastogens. At the current time a survey instrument is being prepared under contract for interviewing mothers and fathers of spontaneous abortuses and controls in order to assess exposure in occupation, household, personal habits, or other vehicles. The disease outcomes of interest are spontaneous abortion, anatomic, or cytogenetic abnormality of fetal tissues.

PROJECT DESCRIPTION

METHODS EMPLOYED: A case-control study is planned in which 500 cases of spontaneous abortion and stratum-matched control will be studied. Through the survey instrument, a medical history and history of exposure will be elicited from both parents. Tissues studied include parental blood and fetal tissue cytogenetics, fetal gross and microscopic pathology, paternal sperm, and an assay for viral agents.

MAJOR FINDINGS AND PROPOSED COURSE: A literature review has been completed and preliminary interview instrument developed. A number of experts in the field have been consulted to solicit expert advice in development of the protocol. A contract is currently being awarded for development and pretesting of the survey instrument to be used. The proposed course of the study would be three years, commencing approximately January 1, 1980.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The response of man to environmental mutagens, particularly via fetal wastage, may be an important health response not only in itself, but also as an indicator of other more serious and longer range damage to the genome. Nonlethal recessive mutation and malignancy are less obviously manifest but may be reflected when short-term results of this type can be demonstrated with a particular exposure. This methodology may therefore serve as a prototype for *in vivo* studies of mutagen exposure which may have implications for more widespread genetic damage and/or neoplasia.

FRED HUTCHINSON CANCER RESEARCH CENTER - Seattle, Washington 98104
LOUISIANA TECH UNIVERSITY - Ruston, Louisiana 71272
(RFP #NIH-ES-77-22)

TITLE: Statistical Development of Multistage Carcinogensis Models

CONTRACTOR'S PROJECT DIRECTORS: Ross L. Prentice, Ph.D. (FHCRC) and
Kenny S. Crump, Ph.D. (LTU)

PROJECT OFFICER (NIEHS): David G. Hoel, Ph.D., Chief, Biometry Branch

DATE CONTRACT INITIATED: May, 1978

CURRENT ANNUAL LEVEL: $61,491 (FHCRC) and $46,113 (LTU)

PROJECT DESCRIPTION

OBJECTIVES: To develop and evaluate statistical procedures for utilizing data from animal tests conducted at high, often maximum tolerated dose rates to estimate carcinogenic risks at very low dose rates, such as might be encountered by humans in their daily environment.

MAJOR FINDINGS AND PROPOSED COURSE: Under the FHRC contract a variety of studies based on proportional hazards and accelerated failure time models will be initiated, including (1) the investigation of various multistage and multipath assumptions leading to tumor incidence rates, (2) the adaptation of the use of cause specific hazard functions and cause specific regression coefficients to the estimation and extrapolation of dose effects in the presence of competing causes of death, and (3) the investigation of the use of censored data generalizations of Wilcoxin and log-rank test statistics in the derivation of estimators of dose effect coefficients.

Under the LTU contract an application of Cox's life table method to the multistage model (for carcinogenesis) will form the basis for extending an existing procedure for analyzing dichotomous data so that time-to-tumor information can be utilized. With this approach hypothesis tests about the shape of the dose response curve at low doses will be developed as well as procedures for obtaining point estimates and confidence intervals for the age-dependent risk of cancer at low dose rates.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In the absence of relevant human data cancer risk estimates extrapolated from laboratory animal experiments will continue to have a major impact on regulatory decision-making policies.

LABORATORY OF ANIMAL GENETICS

THE LABORATORY OF ANIMAL GENETICS
Summary Statement

Last year's Laboratory of Environmental Mutagenesis was recently divided into the Laboratory of Animal Genetics and the Laboratory of Molecular Genetics. The Laboratory of Animal Genetics now consists of four individual research groups, each of which is devoted to problems concerning the effects of environmental mutagens on genetic aspects of human health. In parallel with the central mission of the Laboratory of Molecular Genetics, the Laboratory of Animal Genetics is actively involved in investigations regarding the nature of gene mutations, with particular emphasis on the study of whole-animal (eukaryotic) systems, including the mouse and Drosophila. Strong emphasis has been placed on the future study of gene mutation using the techniques of recombinant DNA.

Besides work directed toward understanding eucaryotic gene mutations, considerable effort has been given to exploring new technologies (such as high-resolution two-dimensional gel electrophoresis) to detect mutationally altered gene products.

The recognition over the last decade of the potential role of environmental chemicals in the etiology of human disease has stimulated considerable interest and growth in the area of environmental mutagenesis. There has been remarkable public awareness of and concern for the importance of protecting the human genome from genetic damage that would constitute a legacy for many future generations. The establishment and development of this laboratory was and continues to show a serious attempt to respond to these needs. Although growth has been steady, it has occurred with continuous re-evaluation of emphasis and direction.

The scope of research that falls within the domain of this laboratory is quite large. On the biochemical side are investigations ranging from basic studies on the structure and function of both normal and mutant proteins in the mouse and Drosophila, to the immunological identification of cross-reacting materials of enzyme mutants carrying non-functioning alleles. Some LAG programs, furthermore, aim towards the development of an understanding of basic genetic mechanisms or fundamental base-line measurements, including investigations into the frequency of deleterious alleles in population and the forces which maintain them there as well as estimations of genic heterozygosity in natural populations.

POPULATION GENETICS

Future regulatory decisions about permissible human exposures to environmental mutagens will have to rest not only upon estimates of the specific mutagenicities of numerous chemicals, but also upon a detailed knowledge of the genetic structure of the exposed population: both sorts of information are required in order to estimate the magnitudes of induced increases in the frequencies of deleterious genes. There remain many areas of ignorance concerning the genetic basis of differences among individuals (in general health, in development, and in both physical and mental abilities), and concerning the degree to which these differences are related to mutation rates. The population genetics group is primarily concerned with gathering base-line information about genetic variation in natural populations. The classical model system for studies of this sort, one which is vastly less expensive and better understood than are humans themselves, is Drosophila. Most of the present research effort is devoted to the study of heritable enzyme deficiencies, which are close analogues of a variety of human biochemical genetic diseases. Extensive surveys of such variation are required in order to provide starting points from which well founded models can be obtained describing the dynamics by which such mutants arise and disappear in populations.

Another project involves measuring the induction of mutationally modified or abolished enzymes by low-level chronic gamma irradiation; it should be noted that such chronic exposure is much more characteristic of real environmental exposures, whether they be radiations or chemicals, than are the acute exposures usually delivered in laboratory experiments. One important result from this study has been the observation that such chronic-irradiation mutation rates are not very different from acute-irradiation mutation rates.

One area of genetics which has been largely ignored in the past by workers in environmental mutagenesis is cytoplasmic genetics; nevertheless, there are important genetic determinants in the cytoplasm. Genetic variation has, therefore, been explored in the mitochondrial DNAs among diverse species of Drosophila, and has been observed to be substantial.

MAMMALIAN GENETICS

The Mammalian Genetics program is primarily concerned with studying the induction, transmission and long-term effects of genetic damage in mammals due to gene mutations and chromosomal aberrations. The chemical mutagen TEM has recently been shown to induce recessive lethal mutations in mice. Some of the heterozygotes are being bred and the offspring tested to compare the transmission of the wild-type and lethal alleles in order to characterize the transmission of the mutations and determine the effects of lethal heterozygosity on factors such as reproductive fitness. Since most of the homozygous recessive lethals act at about the time of implantation in the mouse, studies are being conducted on very early developmental stages. In these experiments two-cell embryos are retrieved from mated females and cultured *in vitro* in order to determine at what stage and in what manner development is disrupted. In another series of continuing studies, the transmission of induced chromosome aberrations (translocations) and their effects on foetal mortality are being investigated. At present, cytogenetic analysis of the size and position of the aberrations on the chromosomes is being conducted in attempts to determine if there is a correlation between these factors and the amount or pattern of foetal mortality produced by the aberration. Studies are also underway on the induction of Sister Chromatid Exchanges (SCE), specifically to extend cytogenetic analysis of SCE's to a variety of tissues in the intact mammal.

This work requires in vivo administration of 5-bromodeoxyuridine and subsequent harvest (after several hours to several weeks) and analysis of chromosomes from various tissues. A number of agents, including known mutagens and carcinogens, are being analyzed to determine whether they induce SCE's. Pilot studies are being conducted in order to extend sister chromatid exchange analyses to embryonic and perinatal tissues.

CHEMICAL GENETICS

The main effort of this research program centers on the sequencing of the primary structure of mammalian lactate dehydrogenase as well as gene 32 protein from T4. With the intensive support and collaboration from the Genetic Enzymology Program, gram quantities of lactate dehydrogenases were purified from mouse tissues. The amino acid compositions, tryptic peptide maps and part of the amino acid sequences have been determined. In the case of lactate dehydrogenases, primary structural data clearly indicate that the subunits A (muscle) and B (heart) appear to be more closely related to each other than to subunit C (testis). Sequential analysis of T4 gene 32 protein reveals unique characteristics of the protein which are important for the binding of DNA.

Sequencing of lactate dehydrogenases, especially LDH-X (a sperm-specific isozyme), is important for on-going work in another NIEHS laboratory directed towards the development of a mutational assay whose endpoint would be alterations in the LDH-X phenotype brought about by amino acid substitutions. A knowledge of the amino acid sequence of the parental form of the enzyme may, therefore, become important in the near future. Furthermore, since a part of the analysis of mutation will involve immunological variation, the sequence analysis will be extended to determining the antigenicity of various fragments of the protein.

GENETIC ENZYMOLOGY

The main objectives of this research group are (1) explorations of new methodologies for the biochemical screening of mutant enzymes or proteins from the offspring of mutagenized animals, and (2) biochemical analyses of natural and induced variants of mouse and Drosophila enzymes. We have attempted to develop two-dimensional gel electrophoresis as a tool for the screening of charge variations of proteins from mouse tissues and Drosophila. The high sensitivity and reproducibility of this technique has allowed us to (1) search for strain variations of proteins from different mouse tissues and from Drosophila, (2) conduct detailed genetic analyses of the identified protein markers, (3) map protein spots on gels against known purified enzymes or proteins, and (4) analyze the possible expression of the inactive proteins corresponding to the null mutants of a given enzyme that has already been mapped on the gels, such as α-GPDH in Drosophila.

To characterize the mutants and variants identified in mice and Drosophila, the following enzymes have been purified and antisera raised: LDH-1, LDH-5, LDH-X, PGK-1, PGK-2, CPK, Me-C, G6PD, PGI and GR from the mouse; and

α-GPDH, PGI c-MDH, s-MDH, ADH and Me from Drosophila. More than a dozen null mutants of Drosophila enzymes have been analyzed immunologically for the expression of cross-reacting materials (CRM). The results of such analysis have strongly supported the interpretation of the nature of γ-irradiation-induced mutations in Drosophila.

During the processes of biochemical screening, a spontaneous mutant of mouse cytoplasmic malic enzyme have been identified and characterized immunologically as well as biochemically. The null allele of this mutant mouse was shown to express no cross-reacting materials. The homozygous null mouse is nevertheless viable. Several enzymes in the related biochemical pathways have been compared with those of the normal mice. No significant changes of these enzyme activities were observed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60042-04 LAG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Study of Recessive Lethal Mutations Induced in Mice by Chemical Mutagens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Sheridan	Research Geneticist	LAG	NIEHS
Other:	R. Dunn	Biologist	LAG	NIEHS
	R. Sorg	Geneticist	LAG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.9	PROFESSIONAL: 0.7	OTHER: 1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies are being conducted of the transmission of recessive lethal mutations which were induced in mice by chemical mutagens. Backcross progeny between offspring of lethal bearing animals and their daughters are used to detect the presence of recessive lethals. An increase in fetal mortality is used as indicator of the lethal. The induction of recessive lethal mutations by a chemical has previously been demonstrated in this project. No adequate study of this class of mutation has been conducted with regard to heterozygous effects. It is our purpose to investigate what might be the mode of action and long-term effects of such mutations on mammals.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Inbred male mice of the CBA strain were treated with a known chemical mutagen (TEM) and mated to untreated females of the same strain. F_1 male progeny were collected and individually mated to females of the strain. A series of backcrosses between F_2 females and their sires were conducted to determine whether there was an increase in fetal mortality indicative of the presence of a recessive lethal. Subsequently, large numbers of offspring were derived from lethal-bearing F_1 males, and are being studied to determine if they have inherited the lethal.

MAJOR FINDINGS AND PROPOSED COURSE: Analyses of the data indicated that a high frequency of the F_1 males studied were bearers of recessive lethals. Comparison of pedigrees give evidence that the greater proportion of these lethals were induced by the mutagenic treatment. Present studies include investigations of the effects of recessive lethals on early developmental stages in homozygotes, and comparisons of heterozygotes with normal homozygotes. In future attempts at biochemical identification of the loci in question will be made.

SIGNIFICANCE TO BIOCHEMICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is estimated that on the average the human genome contains 5 deleterious recessive genes. Although there are known examples of genes which have an extreme effect in the homozygote also showing an effect in the heterozygote, for the most part little is known about the mode of action or long-term effects on the bearer of such genes. Studies are necessary to determine frequencies of induction of this class of genes by mutagenic agents and their consequences to the individual.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60057-03 LAG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Molecular Analyses of Mitochondrial DNAs of Different Species of Drosophila.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Langley	Research Geneticist	LAG	NIEHS

COOPERATING UNITS (if any)

D. Shah, Department of Microbiology, Cummings Life Science Center, Chicago, IL

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have done preliminary characterization of mitochondrial DNAs isolated from three species of Drosophila. Electron microscope heteroduplex and restriction endonuclease analyses have revealed that these mtDNAs have undergone a significant evolutionary divergence in their nucleotide sequences. We are constructing specific cleavage maps of these mtDNAs using various restriction endonucleases. Restriction endonucleases recognize specific base sequences within a DNA molecule and have proven useful in mapping genes and detecting single base-pair substitutions. We have determined the levels of intra-specific variation in the restrictive maps of three species.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Electron microscope heteroduplex analysis and restriction endonuclease cleavage analysis are currently being used to estimate the nucleotide sequence divergence in the circular mitochondrial DNAs of different species of *Drosophila*.

MAJOR FINDINGS AND PROPOSED COURSE: Size heterogeneity has been detected in the mitochondrial DNAs of distantly related species of Drosophila. Electron microscope partial denaturation mapping has revealed the presence of adenine-thymine rich regions in the mitochondrial DNAs of Drosophila. The length of the AT-rich region varies in different species. Heteroduplex analysis has shown the AT-rich region to be the evolutionarily most pliable region in the mitochondrial DNA of Drosophila. Specific cleavage sites of four different restriction endonucleases have been mapped on mitochondrial DNAs of three species of Drosophila. Some important information has been obtained from the restriction endonuclease cleavage maps concerning the evolution of these mitochondrial DNAs. Population genetic variation in the restriction maps of three species has been determined. This variation appears to be similar to that seen in nuclear DNA.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Genetic variation in mitochondrial DNA of higher eukaryotic organisms must be understood in order to judge the consequences of mutational damage. Very little is known about the extent and nature of genetic variation in mitochondrial chromosomes of animals. This project will provide some basic information and hopefully provide some evidence for the role of newly arising mutants.

PUBLICATIONS

Shah, D.M. and Langley, C.H.: Electron microscope DNAs: Evolution of A + T-rich region. *Plasmid* 2: 69-78, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60058-03 LAG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Amount and Effects of Null Allozymic Variation in Natural Populations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Langley	Research Geneticist	LAG	NIEHS
Other:	R. Voelker	Senior Staff Fellow	LAG	NIEHS
	A. Leigh Brown	Visiting Fellow	LAG	NIEHS
	S. Ohnishi	Visiting Fellow	LAG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.3	1.2	2.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☑ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A Raleigh, North Carolina population of Drosophila melanogaster was sampled to determine the frequency of null alleles at 25 allozyme loci. Nulls were found at 13 of 20 autosomal loci with a weighted mean frequency of 0.0025, with a range from 0.00 to 0.01. No nulls were found at five X-chromosome loci. We tentatively suggest that enzyme nulls may be part of the genetic variation contributing to the inbreeding depression observed when chromosomes taken from nature are made homozygous.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Extraction of chromosomes from widely separated natural populations utilizes dominantly marked balancer chromosomes. Electrophoretic assays utilize gel electrophoresis preparation and staining techniques. Cytological analyses involve examination and photography of salivary gland chromosome preparations. Immunologic assays involve preparations of antibodies to purified wild-type enzymes and detection of cross-reacting materials.

MAJOR FINDINGS AND PROPOSED COURSE: A sample of between 800 and 1000 gametes from a Raleigh, North Carolina population of *Drosophila melanogaster* has been screened for nulls at twenty-five allozyme loci. No nulls were found at any of the five X-chromosome loci. The following nulls were found at loci located on the second and third chromosomes: *Got-2*, 3; *a-Gpdh*, 7; *cMdh*, 2; *Adh*, 1; *Dip-A*, 2; *Pgi*, 1; *Hex-C*, 1; *Idh*, 1; *Est-c*, 4; *Odh*, 1; *Men*, 6; *Aldox*, 9; *Acph*, 1. Nulls were not recovered at seven autosomal loci. The weighted mean frequency of nulls at autosomal loci was 0.0025. Qualitative analyses of the nulls have shown that most null-bearing chromosomes also carry a lethal unrelated to the null locus. A number of the nulls have been determined to be CRM-positive. These analyses are continuing. Our finding that many of these nulls are viable and fertile (when separated from the unrelated lethals) lead us to consider the possibility that allozyme nulls may be the biological manifestation of so-called "polygenes," which have hitherto only been described statistically. By analyzing samples from two other populations we hope to learn more about the mechanisms which are responsible for the occurrence of nulls at the low levels at which they are observed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60059-03 LAG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of Chromosomal Aberrations on Embryonic Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Sheridan	Research Geneticist	LAG	NIEHS
Other:	R. Sorg	Geneticist	LAG	NIEHS

COOPERATING UNITS (if any)

H. Michelmann, University of Göttingen, West Germany

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS,NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project is designed to study the effects of chromosomal aberrations on fetal mortality and development. Comparisons are made between in vivo effects and effects on mouse embryos grown in culture. Chemical mutagens are known to be capable of inducing chromosomal aberrations such as translocations which may be transmitted to the next generation. Increased rates of fetal death are usually observed among offspring to translocation heterozygotes. It is our purpose to investigate the rates of transmission of such aberrations, and the processes of fetal mortality by direct observation of the development of embryos.

PROJECT DESCRIPTION

METHODS EMPLOYED: Known translocation-bearing mice are mated and the F_1 sons studied to determine the frequency of translocation heterozygotes. In other matings the females are euthanized shortly after fertilization, and two cell embryos are retrieved from the oviducts and established in culture. Stage of development arrest is determined.

MAJOR FINDINGS AND PROPOSED COURSE: F_3 males are currently being analyzed to determine whether they are translocation heterozygotes. Cytogenetic analysis is being conducted in attempts to correlate the nature of the aberration and the effect on embryonic development.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Chromosome aberrations in man are known to cause disturbances in development of embryos leading to death of the embryo or to abnormal offspring. An understanding of the fate and effects of chromosome aberrations which may be caused by environmental agents is necessary for evaluation of the possible risks such induced aberrations may imply for man.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60062-03 LAG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Molecular Characterization of Isozymes and Mutant Enzymes in Mammals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Li	Research Geneticist	LAG	NIEHS
Other:	S.-M. Chang	Visiting Fellow	LAG	NIEHS
	S. Huang	Visiting Fellow	LAG	NIEHS
	C.-Y. Lee	Senior Staff Fellow	LAG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION
NIHES, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The amino acid composition and tryptic peptide maps of three isozymes, LDH-M, LDH-H and LDH-X, purified from mouse muscle, heart and testis, respectively, have been compared. The testicular isozyme LDH-X has also been purified from both rat and hamster, and its amino acid composition determined in each case. Isozyme LDH-M has also been purified from horse muscle, and its enzymatic properties, amino acid composition and tryptic peptide map determined.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Lactate dehydrogenases are purified by AMP-affinity chromatography. Tryptic peptide maps are analyzed on cellulose thin-layer plates by electrophoresis and chromatography.

MAJOR FINDINGS AND PROPOSED COURSE: Three homotetrameric lactate dehydrogenase isozymes, LDH-M (A_4), LDH-H (B_4), and LDH-X (C_4), have been purified from mouse muscle, heart and testis, respectively. Based upon the amino acid composition and tryptic peptide maps of three isozymes, subunits A (muscle) and B (heart) appear to be more closely related to each other than to subunit C (testis). Two other testicular isozymes, LDH-X, have been purified from rat and hamster, and their amino acid composition and tryptic peptide maps are being compared. The primary structure variation of these testicular isozymes will be correlated with their antigenic properties. Isozyme LDH-M has also been purified from horse muscle. The enzymatic properties, amino acid composition and tryptic peptide map have been determined and compared with those of other mammalian LDH isozymes.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Primary structure characterization of various lactate dehydrogenase isozymes indicates the evolutionary relationship of different LDH genes. Molecular analysis of mutant proteins will elucidate the nature of genetic mutations in mammals.

PUBLICATIONS

Chang, S. M., C.-Y. Lee, R. Chen, and S. Li: Compositional relatedness of lactate dehydrogenase isozymes from mouse muscle, heart, and testis. Federation Proceeding 37: 1799, 1978.

Huang, S., C.-Y. Lee, and S. S.-L. Li: Purification and partial characterization of M4 lactate dehydrogenase isozyme from horse muscle. Int. J. Biochem. 10: 279-284, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-ES 60063-03 LAG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Amino Acid Sequence of Mouse Sperm Lactate Dehydrogenase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.	Li	Research Geneticist	LAG	NIEHS
Other:	J.	Marciniszyn	Senior Staff Fellow	LAG	NIEHS
	Y.-C.	Pan	Visiting Fellow	LAG	NIEHS
	S.	Huang	Visiting Fellow	LAG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.8	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The sperm-specific lactate dehydrogenase from mouse has been cleaved into small peptides by CNBr and trypsin. These peptides are being purified by gel filtration and ion-exchange chromatography. Amino acid sequences of some purified peptides are being determined. Thus far, two-thirds of the 330 residues have been sequenced.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Sperm-specific lactate dehydrogenase from mouse testis has been purified by AMP-affinity chromatography, and fragmented into peptides by chemical (CNBr) and enzymatic (trypsin) cleavage. These peptides are being separated by gel filtration and ion-exchange chromatography. Amino acid sequences of the pure peptides are being determined by automatic Edman degradation on a liquid-phase protein sequencer.

MAJOR FINDINGS AND PROPOSED COURSE: The CNBr fragments and tryptic peptides of mouse LDH-X are being separated and the amino acid sequences of some pure peptides are being determined. Thus far, two-thirds of the 330 residues have been sequenced. The complete primary structure will be established by overlapping various peptide sequences. The submolecular fragments of this LDH-X will also be used to study the antigenic properties of this enzyme.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The complete amino acid sequence of this sperm-specific lactate dehydrogenase from mouse will be correlated with the results obtained from X-ray studies regarding the structure-function relationship of this enzyme. Elucidation of the primary structure of this enzyme is essential for studies of the antigenic properties of lactate dehydrogenases. This sperm-specific enzyme will be used for monitoring of mutations caused by environmental mutagens in human populations.

PUBLICATIONS

Huang, S., Y.-C. E. Pan, J. P. Marciniszyn, Jr., and S. S-L. Li: Amino acid sequences of the substrate and coenzyme binding sites from mouse testicular lactate dehydrogenase isozyme. Federation Proceedings 38: 324, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-ES 60064-03 LAG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Primary Structure Characterization of Mouse Muscular Lactate Dehydrogenase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Y. Nakashima	Visiting Scientist	LAG	NIEHS
Other:	S. Li	Research Geneticist	LAG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.3	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Lactate dehydrogenase from mouse muscle has been fragmented into small peptides by chemical and enzymatic procedures. These peptides are being purified by gel filtration and ion-exchange chromatography. Amino acid sequences of some pure peptides are being determined. Thus far, more than one third of the 330 residues have been sequenced.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Lactate dehydrogenase from mouse muscle has been purified by affinity chromatography. The purified enzyme has been cleaved into small peptides by chemical (CNBr) and enzymatic (trypsin and *Staphylococcal* protease) methods. These peptides are being isolated by gel filtration and ion-exchange chromatography. Amino acid sequences of pure peptides are being determined with an automatic protein sequencer.

MAJOR FINDINGS AND PROPOSED COURSE: The CNBr fragments of this enzyme (LDH-M) are separated by gel filtration. Some pure peptides have been sequenced. The small peptides derived from protease digestion are being purified. The amino acid composition of the pure peptides is being determined. Thus far, more than one-third of the 330 residues have been sequenced. The total amino acid sequence of LDH-M will be established from several overlapping peptide sequences.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The amino acid sequence differences among various isozymes of lactate dehydrogenases will be correlated with their antigenic properties. This information is essential for a genetic monitoring program using monospecific antibodies of the sperm-specific enzyme as a mutation marker.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60087-02 LAG

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Biochemical Studies of Mouse Enzymes, II. PGK-B Variants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.-Y. Lee	Senior Staff Fellow	LAG	NIEHS

COOPERATING UNITS (if any)

B. Pegoraro, Duke University, Durham, North Carolina

LAB/BRANCH

Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Three genetic variants of 3-phosphoglycerate kinase-B (a sperm-specific isozyme) were purified respectively from DBA/2J, C3H/HeJ and C57BL/6J mice, and their kinetic properties were compared. Stability of the enzymes was compared at high temperatures, extreme pH's or in the presence of urea and iodoacetate.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Purification of the sperm-specific isozyme from three different strains of mice by affinity chromatography on 8-(6-aminohexyl)-amino-ATP-Sepharose, according to the method developed previously in this laboratory.

MAJOR FINDINGS AND PROPOSED COURSE: Three PGK-B variants examined were found different in two major aspects: the PGK-B variants from DBA/2J and C3H/HeJ mice have very similar kinetic properties, but differ in stability at high temperatures. However, the variant from C57L/J mice is thermally stable but has a low specific activity. In order to clarify the nature of the variation, it is proposed to employ immunlogical techniques to determine if the low activity variant is the result of a structural or a regulatory mutation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A knowledge of mouse biochemical genetic variants from a biochemical point of view is crucial for mutagenesis testing in mammals.

PUBLICATIONS

Pegoraro, B., and Lee, C.-Y.: Purification and characterization of two isozymes of 3-phosphoglycerate kinase. *Biochem. Biophys. Acta*. 522: 423-433, 1978.

Lee, C.-Y. and Pegoraro, B.: Biochemical and immunological characterization of three genetic variants of 3-phosphoglycerate kinase-2. *Biochem. Genetics*, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60091-02 LAG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Biochemical Studies of Mouse Enzymes and Genetic Variants, I. Phosophoglucose Isomerase (PGI)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. Charles	Visiting Fellow	LAG	NIEHS
Other:	C.-Y. Lee	Senior Staff Fellow	LAG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.9	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Genetic variants of phosphoglucose isomerase were purified from muscle extracts of DBA/2J and C57BL/6J mice. Structural and kinetic properties of these two electrophoretic variants were compared under different experimental conditions in an attempt to find any other biochemical differences.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: A C8-ATP-Sepharose column was employed as a major step of enzyme purification by "affinity elution."

MAJOR FINDINGS AND PROPOSED COURSE: These two electrophoretic variants were found to have a thermal stability difference at 50°. The half-life for the DBA/2J and C57BL/6J enzymes were shown to be 3 min and 1 min, respectively. However, they have identical kinetic parameters and structural characteristics.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This is part of our overall program on the development of methodology for biochemical screening of enzyme variants or mutants in mice.

PUBLICATIONS

Charles, D. and Lee, C.-Y.: Biochemical and immunological characterization of genetic variants of phosphoglucose isomerase from mouse. Biochem. Genetics, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60092-02 LAG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Biochemical Mutations in Drosophila Induced by Chronic Irradiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Langley	Research Geneticist	LAG	NIEHS
Other:	R. Voelker	Senior Staff Fellow	LAG	NIEHS
	R. Racine	Visiting Fellow	LAG	NEIHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An analysis of mutants recovered after 1000 lines of a balanced lethal strain of Drosophila melanogaster were exposed to chronic low-dose (8.7 rad/hr) γ-irradiation was carried out. The following mutants were recovered: Got-2, 1; α-Gpdh, 6; cMdh, 2; Adh, 0; Dip-A, 0; Hex-C, 4; α-Amy, 0. These represent 7 independent mutation events. Only one of these was associated with a chromosomal aberration (deficiency). Of five which could be analyzed for CRM production, three were CRM-positive. These results suggest that the majority of mutants induced by low-dose-rate γ-irradiation are point mutations.

PROJECT DESCRIPTION

METHODS EMPLOYED: A strain of *Drosophila melanogaster* composed of balanced lethal heterozygotes, SM1/$+_{\ell}$, was replicated into 1000 lines and brother-sister mated each generation while being exposed to low-dose-rate γ-radiation (8.7 rads/hour) for 15 generations (a cumulative dose of 43848 rads). This strain is heteroallelic for the soluble enzyme loci *Got-2*, *α-Gpdh*, *c-Mdh*, *Adh*, *Dip-A*, *Hex-C* and *α-Amy*. When electrophoresed after the irradiation, the unmutated flies give codominant phenotypes. If one of the alleles in a particular line has sustained a null mutation (loss of enzyme activity) the phenotype will appear dominant (or homozygous).

MAJOR FINDINGS AND PROPOSED COURSE: 13 mutations were found at four different loci: 6 *α-Gpdh*, 4 *Hex-C*, 2 *cMdh*, and 1 *Got-2*. Seven of the mutants are of independent origin and are apparent nulls. Three mutants (*α-Gpdh*, 2 *cMdh*) produce gene products and are probably point mutations. Four mutants, (2 *α-Gpdh*, *cMdh* and *Got-2*) produce no functional hetero- or homodimers as detected from gel staining.

The mutant salivary gland chromosomes were also analyzed. Of the 7 independent mutants, none appear to be associated with a chromosome aberration. One *α-Gpdh* null does, however, appear to be associated with a genetic deficiency which is too small to be observed cytologically.

This experiment suggests that with chronic low-dose-rate irradiation, the proportion of cytogenetic deletions is low. In order to get an estimate of the mutation rate induced by chronic low-dose-rate γ-radiation, a recessive-lethal experiment was carried out for the X and 2nd chromosomes. One generation (from egg to adult fly) was chronically irradiated at a low dose rate (8.4 rads/hour) for 14 days (a cummulative dose of 2923 rads). Induced recessive lethal rates for the X and 2nd chromosomes of 3.7% and 11.7%, respectively, were found. This recessive lethal experiment indicates that chronic γ-radiation leads to a similar number of mutants per rad as acute irradiation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These findings suggest that the majority of mutations induced by chronic low dose-rate radiation are point mutations rather than chromosomal aberrations.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60094-02 LAG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

BrdU-Dye Studies of Meiotic Recombination

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. Allen	Staff Fellow	LAG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.7	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In vivo 5-bromodeoxyuridine (BrdU)-dye methodology is utilized for a variety of cytogenetic studies in rodent germ cells Analyses of spermatogonial DNA replication and sister chromatid exchange (SCE) patterns are accomplished, and meiotic exchanges are visualized in 1° and 2° spermatocytes. Together, these studies can form a system for interpreting details of meiotic recombination. Mutagen effects upon SCE and/or meiotic exchange will be examined.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: *In vivo* approaches are used in a rodent system to allow for detection of BrdU in various germ cell stages. Methods based upon administering BrdU by serial injections, or by tablet implantation, provide for continuous exposure of replicating spermatogonial cells to analogue. Detection of BrdU in spermatogonial or spermatocyte stages is accomplished with differential staining in accordance with Hoechst fluorescence or Hoechst + Giemsa techniques.

MAJOR FINDINGS AND PROPOSED COURSE: Meiotic exchanges can be visualized in a mammlaian system, and these can be studied in regard to various characteristics of crossing over. Future studies will include analysis of perturbations of meiotic recombination from various exogeneous agents.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Meiotic recombination is a principle source of genetic variability; yet, it is poorly understood at the cytological as well as the molecular level. Very little is known about possible drug effects on this process. Cytogenetic analyses of spermatocyte cross-over events, using high resolution BrdU-dye techniques, can provide new information regarding germ cell DNA synthesis and exchange. In a mammalian system, this approach can be used to examine possible drug effects on SCE and meiotic exchange processes.

PUBLICATIONS

Allen, J.W., Shuler, C.F., and Latt, S.A.: Bromodeoxyuridine tablet methodology for *in vivo* studies of DNA synthesis. *Som. Cell Genet.* 4: 393-405, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60095-02 LAG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Sister Chromatid Exchange Analyses in Rodent Fetal Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Allen	Staff Fellow	LAG	NIEHS
Other:	M. Sanyal	Reproductive Physiologist	LET	NIEHS
	E. El-Nahass	Visiting Fellow	LET	NIEHS

COOPERATING UNITS (if any)

Laboratory of Enivornmental Toxicology, NIEHS

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.3	PROFESSIONAL: .7	OTHER: 0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In vivo sister chromatid exchange (SCE) analyses have been extended to rat fetal as well as maternal tissues. SCE induction effects from selected mutagens/teratogens are being examined after maternal drug metabolism and transplacental passage to various fetal tissues. In some instances, in vitro methodology is also employed in order to study direct effects from the parent compound, and also from known metabolites. The system should allow for sensitive cytological assessment of comparative drug damage at various maternal-fetal interaction stages.

PROJECT DESCRIPTION

METHODS EMPLOYED: Subcutaneous BrdU tablet implantation in pregnant rats is used to effect analogue substitution in maternal and fetal tissues. Sister chromatid differentiation and exchange analysis is then carried out after Hoechst or Hoechst-and-Giemsa staining of chromosome preparations from various tissues. SCE increments are detectable after drug exposures during *in vitro* or *in vivo* DNA replication periods.

MAJOR FINDINGS AND PROPOSED COURSE: Experimental conditions have been worked out for effecting sister chromatid differentiation in multiple fetal tissues within an *in vivo* setting. The system, inclusive of *in vitro* components, permits assessment of direct drug damage, or damage after various physiological influences in regard to metabolism and placental transfer of the drug.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: *In vivo* analyses of SCE permit sensitive detection of genetic damage by drugs subject to host activation or detoxification. Comparative studies of SCE induction in maternal and fetal tissues should afford an estimation of tissue-specific sensitivity to the test drug. Various mutagens/teratogens with action at critical fetal development stages may be examined.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60096-02 LAG

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Comparison of Rates of Induction of Point Mutations and Chromosome Aberrations in Cultured Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Sheridan	Research Geneticist	LAG	NIEHS
Other:	R. Sorg	Geneticist	LAG	NIEHS

COOPERATING UNITS (if any)

D. Clive, Burroughs Wellcome, Research Triangle Park, North Carolina

LAB/BRANCH

Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.1	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to assess the utility of various test systems, different aspects of their potential should be explored. If one type of genetic damage is more easily detected within a system, this might be the criterion of choice. Using the Mouse Lymphoma cell line L5178Y, mutations at the TK locus were induced by chemical treatment. Studies utilizing the same mutagens are conducted on the frequencies of induction of chromosomal damage and aberrations, and compared with the point mutation frequency.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard methods of culture of L5178Y Mouse Lymphoma cells are employed. The chemical treatments are applied in the standard manner. Cytogenetic preparations are made by standard techniques.

MAJOR FINDINGS AND PROPOSED COURSE: Analysis to date indicate that there is a correlation between the induction of point mutations and the induction of chromosomal aberrations. Whether this correlation is coincidental or whether there is a real relationship is currently being considered. Studies include cytogenetic analysis of specific mutant cell lines recovered in the point-mutation studies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The need for efficient biological detectors of genetic effects from environmental mutagens can be filled only when the detection system has been validated. Such validation can to some extent be arrived at by using several different genetic end points, and comparing their efficiencies, so that information regarding the basic mechanisms and relationships underlying induction of genetic damage may be deduced.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 61001-01 LAG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Sister Chromatid Exchange Studies in Dividing Cells of the Rodent Colon.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Roberts	Visiting Fellow	LAG	NIEHS
Other:	J. Allen	Staff Fellow	LAG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We propose to develop an in vivo system which would enable the examination of sister chromatid exchanges (SCE) in cells of the colonic epithelium of rodents. Methodology needs to be worked out so that dividing cells from deep within the Crypts of Lieberkuhn can be obtained in sufficient numbers. 5-Bromodeoxyuridine (BrdU)-dye techniques will be used to visualize both baseline and drug-induced SCE.

PROJECT DESCRIPTION

METHODS EMPLOYED: A number of physical and chemical manipulations are being tried in order to determine the conditions which produce the greatest number of the target cells. Following this, various procedures for effecting BrdU incorporation will be tested and the timing of administration of the analogue will also be varied. Hopefully, a method for obtaining good numbers of metaphase cells showing sister chromatid differentiation will result.

MAJOR FINDINGS AND PROPOSED COURSE: Experimental conditions have been worked out which do permit the release of many dividing cells. To visualize sister chromatid differentiation, BrdU will be administered by various routes and at various times prior to harvest of cells. Following the establishment of a reliable protocol, baseline and drug-induced SCE levels will be examined.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Tumors of the colon are one of the most common forms of neoplasia in the Western World, and in fact, several agents specifically induce colonic tumors. Development of the techniques mentioned here may provide a model for the study of the kinds of lesions induced by carcinogens in the gastro-intestinal tract. It may also be possible to study the kinetics of cell division in the intestine.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 61002-01 LAG

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Induction of Sister Chromatid Exchanges (SCE) by Ethyl Carbamate in Various Mouse Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G.Roberts	Visiting Fellow	LAG	NIEHS
Other:	J. Allen	Staff Fellow	LAG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.7	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Differences in baseline and drug-induced frequencies of SCE between somatic and germ cells have been demonstrated. This capability of the SCE test was utilized to determine the relative sensitivity of various mouse tissues to the carcinogen ethyl carbamate. 5-Bromodeoxyuridine (BrdU)-dye methodology was utilized for examining SCE in vivo and in vitro. The extent of SCE induction in the tissues used correlates well with their known sensitivity to this agent with respect to the induction of carcinogenic and mutagenic endpoints.

PROJECT DESCRIPTION

METHODS EMPLOYED: Cells from mouse bone marrow, liver and spermatogonia were examined for SCE induction by ethyl carbamate. 5-Bromodeoxyuridine (BrdU) was incorporated into the chromosomes of the former two tissues by the subcutaneous implantation of a slowly dissolving tablet of the analogue. Recently hepatectomized mice were used if liver was to be studied. The incorporation of BrdU into spermatogonial cells was effected by means of a series of intraperitoneal injections. In addition, bone marrow cells were cultured in the presence of BrdU in order to confirm the negative effects of ethyl carbamate in vitro.

MAJOR FINDINGS AND PROPOSED COURSE: Each of the tissues responded to ethyl carbamate exposure in vivo with a dose-dependent increase of SCE; however, there were large discrepancies in the extent of this response. Ethyl carbamate is widely and evenly distributed in the mouse; therefore, variable metabolism between tissues or differential sensitivity to the active compound most likely accounts for the observed effects. The tissue responses in order of decreasing sensitivity were as follows: liver, bone marrow, and spermatogonia. Previous negative reports for SCE induction in vitro were confirmed. Further studies with agents of different types and mechanisms of action are required to fully understand the relevance of differential tissue sensitivities.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The analysis of SCE induction in vivo is a highly relevant test system as the metabolic, distribution and excretory systems of the host are intact. This study exemplifies the greater usefulness of in vivo testing in a number of tissues as compared to in vitro studies. Together with further studies of this nature, we may learn more about the factors involved in differential tissue sensitivities to carcinogen/mutagen insult.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 61003-01 LAG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Biochemical Analyses of Drosophila Null Mutants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.-Y. Lee	Senior Staff Fellow	LAG	NIEHS
Other:	D. Charles	Visiting Fellow	LAG	NIEHS
	D. Bronson	Student Employee	LAG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.8	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Six Drosophila enzymes including alcohol dehydrogenase, cytoplasmic and mitochondrial malate dehydrogenase, phosphoglucose isomerase and malic enzyme were purified by affinity chromatography. The null mutants of these enzymes were characterized immunologically in terms of the presence of cross-reacting materials (CRM).

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Affinity chromatography, immunoelectrophoresis.

MAJOR FINDINGS AND PROPOSED COURSE: Two γ-irradiation-induced malate dehydrogenase null mutants were shown to be CRM positive. Two natural null variants of a malic enzyme were determined to be CRM negative

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Characterization of Drosophila null mutants in terms of CRM is an important step towards the understanding of the nature of gene mutation.

PUBLICATIONS

Lee, C.-Y., D. Charles and D. Bronson: Biochemical analyses of null variants of Drosophila enzymes. *J. Biol. Chem.*, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 61004-01 LAG

PERIOD COVERED
October 1, 1978 to September 1, 1979

TITLE OF PROJECT (80 characters or less)

Surveys of Genetic Variation Utilizing Two-Dimensional Eelctrophoresis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Langley	Research Geneticist	LAG	NIEHS
Other:	R. Voelker	Senior Staff Fellow	LAG	NIEHS
	A. Leigh Brown	Visiting Fellow	LAG	NIEHS
	R. Racine	Visiting Fellow	LAG	NIEHS
	S. Ohnishi	Visiting Fellow	LAG	NIEHS
	S. Smith	Student	LAG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Animal Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 2.3	PROFESSIONAL: 2.3	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two-dimensional electrophoresis as described by O'Farrel in 1975 has been applied to the identification and estimation of genic variation in various animal species. After surveying two Drosophila species, mice and humans for electrophoretic variation in the most abundant proteins it can be concluded that previous estimates of the naturally occurring levels were overstated.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Two dimensional electrophoresis was carried out on either whole Drosophila or kidney tissue from mice or autopsied humans. Two species of Drosophila, various laboratory and wild populations of mice and twenty-five humans were surveyed for electrophoretic variation in the most abundant proteins. In the case of Drosophila and mice, the genetic variation was analyzed in crosses to demonstrate the modes of inheritance and linkage relationships.

MAJOR FINDINGS AND PROPOSED COURSE: The general conclusion of these studies is that the level of variation revealed by two-dimensional electrophoresis is less than that observed by earlier allozyme/isozyme techniques. Although there may be some sensitivity differences between the techniques, it seems more likely that biases in the selection of loci surveyed in previous studies accounts for the discrepancy. The survey of genetic variation with two-dimensional electrophoresis will be concluded this year with some investigations of rates of molecular evolution in Drosophila and mice.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The estimation of the standing levels of genetic variation and the understanding of their relationship to changes in the mutation rate is fundamental to the evaluation of public health risk.

PUBLICATIONS

Leigh Brown, A.J. and Langley, C.H.: Reevaluation of level of genic heterozygosity in natural population of *Drosophila melanogaster* by two-dimensional electrophoresis. Proc. Nat'l. Acad. Sci., USA. 76: 2381-2384, 1979.

Racine, R.R. and Langley, C.H.: Genetic analysis of protein variation in *Mus musculus* using two-dimensional electrophoresis. *Biochem. Genet.*, in press.

BROOKHAVEN NATIONAL LABORATORY - Upton, New York
(Interagency Agreement)

TITLE: Chemical and Environmental Mutagen Studies Utilizing the Tradescantia Test System

CONTRACTOR'S PROJECT DIRECTOR: Jack Van't Hof, Ph.D.

PROJECT OFFICER (NIEHS): F. J. de Serres, Ph.D.
Associate Director for Genetics

DATE CONTRACT INITIATED: June 6, 1972

CURRENT ANNUAL LEVEL: $422,000

PROJECT DESCRIPTION

OBJECTIVES: The primary objective of this program is to determine the feasibility of using the Tradescantia stamen hair system to detect the mutagenicity of gaseous and particular air pollutants. Tradescantia was chosen because of the breath of radiobiological data on the somatic mutation system, the versatility and relative ease of handling experimental material, high sensitivity to both chemical and physical mutagens and its small number (12) of large chromosomes suitable for cytological analysis. Moreover, it can be exposed directly to ambient air pollutants.

METHODS EMPLOYED: To exploit the Tradescantia system in pursuit of the above objectives, work has progressed in three main areas; definition of the basic genetics of Tradescantia, elucidation of the mechanisms responsible for mutation-induction and field exposures to ambient air pollution.

Genetic and plant breeding studies are continuing in an effort to clarify the genetic basis of the flower color marker and to develop new clones with improved characteristics.

The Tradescantia system is being utilized in applied studies (testing of new compounds) in both laboratory and field exposures although the major effort during this December to June period has been on field studies. New chemicals are being screened individually for mutagenicity under laboratory conditions and facilities are being prepared to explore the possibility of synergistic reactions between mutagens. Applied studies have been extended into the field through the use of a Mobile Monitoring Vehicle (MMV) which has been fabricated and is undergoing field testing in high pollution areas throughout the country. Pollution site selection and field exposures are being carried out in collaboration with EPA.

MAJOR FINDINGS AND PROPOSED COURSE: Genetic and plant breeding studies are continuing in an effort to clarify the genetic basis of flower color changes in the stamen hair and petal cells of Tradescantia and to develop new clones with improved characteristics.

The genetic studies include several projects related to the genetics on the inheritance of white, pink and blue flower color as well as the genetic analysis of the particular alleles used in the heterozygous clones 02 and 4430.

The plant breeding studies are designed to evaluate the performance of a newly selected dwarf heterozygote in the Mobile Monitoring Vehicle. In addition, new loci are being incorporated into standard clones to extend the text system to include both somatic and genetic mutations for screening. A particularly important project in this area is the extablishment of new clones better adapted for experimental mutagenesis than the present clones of 02 and 4430.

Major emphasis during the past year has been placed on field trials using the Mobile Monitoring Vehicle. Sites visited in the period December 16, 1976 to June 15, 1978 include Elizabeth, NJ, Charleston, WV, Birmingham, AL and Baton Rouge, LA. The results of the four trials indicate that the Tradescantia stamen hair system has shown a positive response to ambient air in Elizabeth, Charleston and Baton Rouge but not in Birmingham. Mutagenic response may be correlated loosely with ambient pollution from petroleum and chemical processing but not steel smelting. No other correlations can be made until data from other sites and validated pollution measurements (to be provided by the EPA Mobile Monitoring Unit) are available.

In view of the successful completion of the development and validation phases of this project, NIEHS will terminate funding at the end of FY79. It is anticipated that the Department of Energy and/or other federal agencies involved in environmental monitoring will continue the operation of the Mobile Monitoring Vehicle for detecting mutagenic air pollutants.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

The Tradescantia stamen hair system is the most sensitive assay system which has been developed to detect the mutagenic activity of environmental chemicals with regard to the production of gene mutations. The simplicity of both the exposure procedure and the scoring of stamen hairs to determine mutation frequency rates is expecially well suited for both basic studies as well as field applications for monitoring air pollutants in general or highly specific areas (i.e. monitoring air pollutants in industries producing biologically active chemicals). The sensitiviity of this assay system makes it especially well suited for monitoring air pollutants as they actually occur and avoids the complexities associated with collecting condensates and evaluation of eluted samples from the condensates. Evaluation of ambient air pollutants is essential to evaluate possible synergistic effects between chemicals which may or may not be biologically active per se.

PUBLICATIONS

McNulty, P. J., Nauman, C. H., Sparrow, A. H., Schwemmer, S. S. and Schairer, L. A.: Influence of x-ray dose fractionation on the frequency of somatic mutations induced in Tradescantia stamen hairs. Mutation Research. 44: 235-246, 1977.

UNIVERSITY OF BRITISH COLUMBIA - Vancouver, British Columbia
(263-77C-0604CC)

TITLE: Testing Compounds for Mutagenic Activity in a Meiotic Non-disjunction Test in Neurospora crassa

CONTRACTOR'S PROJECT DIRECTOR: A. J. F. Griffiths, Ph.D.

PROJECT OFFICER (NIEHS): F. J. de Serres, Ph.D.
Associate Director for Genetics

DATE CONTRACT INITIATED: June 16, 1975

CURRENT ANNUAL LEVEL: $25,000

PROJECT DESCRIPTION

OBJECTIVES: The objective of this contract is to assay environmental chemicals for mutagenic activity in a meiotic non-disjunction test in Neurospora crassa.

METHODS EMPLOYED: The contractor has developed two strains of Neurospora crassa with a series of genetic markers in each on Linkage Group I (LGI). When these two strains are crossed to each other and ascospores are plated (about 10^6 ascospores per cross) on minimal medium, the only ones that can grow are disomic for LGI. The aneuploid condition is unstable and breaks down to form a heterokaryon composed of two (haploid) nuclear types representing the original disomic pair of chromosomes. This heterokaryon is called a pseudowild-type and segregates vegetatively into the two components; thus, pseudowild-type frequency is an index of the frequency of meiotic non-disjunction involving LGI in this organism.

MAJOR FINDINGS AND PROPOSED COURSE: Control platings (untreated crosses) have shown that spontaneous non-disjunction frequencies are quite constant with an average value of 4.9×10^{-5}. Treatment is made by adding test chemicals to the crossing medium. All stages of meiosis will be affected. After plating ascospores from control and treated crosses, a comparison is made of pseudowild-type frequencies. A total of 10 chemicals have been found in control crosses: p-fluorophenylalanine, trifluralin, trimethoprin, caffeine, atrazine, resorcinol, stilphosterol, 2,4-diaminoanisole S, alloxan, 8-hydroxyquinoline. In addition, gamma rays were also found to give a positive test.

Experience with this assay system has shown that it can provide a useful assay for non-disjunction. Statistical analysis of the data indicates that at least five tests have to be performed on each compound in order to obtain statistically reliable data. A single technician under the supervision of the Project Director can perform about 125 tests per year or, with 5 tests per compound, can test 25 chemicals per year.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

In order to develop a truly comprehensive battery of tests for mass screening programs, we must be able to detect all classes of genetic alterations. No such assay exists for non-disjunction. Because of this we only have scanty evidence of chemicals which will cause non-disjunction. The assays with Neurospora have detected activity in 10 chemicals out of 62 tested. This is an important class of genetic alteration with regard to genetic disease in man.

Because of the marked specificity of some chemical mutagens, it is considered essential to continue work with the Neurospora assay to evaluate its utility and feasibility as an assay system in mass screening programs.

PUBLICATION

Griffiths, A. J. F., and Delange, A. M.: p-Fluorophenylalanine Increases Meiotic Non-Disjunction in a Neurospora Test System. Mutation Research 46: 345-354, 1977.

Griffiths, A. J. F.: A Neurospora Prototrophic Selection System for Studying Aneuploidy Production. Environmental Health Perspectives, Vol. 31, August 1979.

LABORATORY OF BEHAVIORAL AND NEUROLOGICAL TOXICOLOGY

LABORATORY OF BEHAVIORAL AND NEUROLOGICAL TOXICOLOGY
Summary Statement

The scientific efforts of the Laboratory of Behavioral and Neurological Toxicology (LBNT) are directed toward the understanding of the behavioral and neurological effects produced by toxic substances. Special emphasis is placed upon the changes in behavior and neurologic function produced by long-term exposure to low levels of a wide variety of chemical and physical agents present in the environment and to exposure during the development of the nervous system.

The goals of the Laboratory are (1) to identify laboratory procedures useful in assessing the role of environmental factors in the development of behavioral and neurological abnormalities, (2) determine the mechanism(s) whereby environmental factors produce their behavioral and neurotoxic effects, and (3) identification of conditions which predispose individuals to the behavioral and neurotoxic effects of environmental factors.

The research goals of the Laboratory are supported by both intramural scientists and contracted research. The scope of the overall effort is broad involving national and international programs and adjunct appointments. Training opportunities exist for graduate students at nearby universities, foreign and American postdoctoral scientists, and American scientists on sabbatical leave. Current expertise is in the areas of behavior and neurochemistry.

BEHAVIORAL TOXICOLOGY

The research of this group is devoted largely to the identification of laboratory procedures useful in assessing the effects of environmental factors on behavioral and neurological function, in determining conditions which predispose individuals to the behavioral and neurotoxic effects of these factors with increasing emphasis on assessing the consequences of exposure to environmental agents during the development of the nervous system.

Group scientists have developed a simple test battery for the neurobehavioral assessment of potential neurotoxins both in adult and young animals. The tests include gross observations of sensory/motor functioning, hindlimb extensor response, forelimb grip strength, startle responsiveness, tremor, performance on an inclined screen, visual placement, rectal temperature, spontaneous motor activity, and measures of acquisition and retention of a learned response. These tests are now being standardized and validated using compounds with known neurotoxic effects.

The effects of polybrominated biphenyl compounds in rats and mice are also being investigated. These compounds have been recognized as an environmental hazard following their inadvertent mixture to animal feed. Rodents exposed to these compounds in their feed show a decrease in body weight, depressed open-field activity, lower forelimb grip strength, and depressed reflexes. An apparent loss of visual placement and the occurrence of hypothermia were noted in some animals. These studies have demonstrated effects of polybrominated biphenyls in rodents similar to those reported to occur in humans thus leading some credence to the utility of the test battery. Neurophysiological and neurochemical studies are planned.

Group scientists are also investigating the neurobehavioral toxicity of acrylamide in rats. Acrylamide is a known neurotoxin having effects generally most easily seen as peripheral neuropathy. This compound is being studied in order to more clearly define the validity of the simple test battery. As expected, deficits in hindlimb function have been found with doses which do not affect forelimb strength further illustrating the usefulness of the two test procedures developed to measure these functions.

In addition, the effect of acrylamide on motor responses under control of operant schedules is being investigated. Acrylamide is also being studied for its effects on peripheral sensation using an operant titration schedule. In this procedure, the rat "informs" the investigator of the point it preserves a shock to its tail by poking its nose in a tube containing a photoelectric cell detector. Other studies with acrylamide are in progress concerning its effects on food and water consumption, body weight, and spontaneous activity of rats.

A study was conducted in which changes in neurobehavioral functioning of rats exposed to carbon disulfide by inhalation was assessed. It was found that hindlimb extensor responses and performance on an inclined screen (motor coordination) were impaired after six weeks of exposure to 2 mg carbon disulfide/l of air for four hours per day.

Several studies are in progress or being initiated concerning the neurobehavioral toxicity of agents administered prenatally, neonatally, or both. These studies are being conducted in collaboration with other laboratories at NIEHS and/or the local universities. One such project concerns the neurobehavioral toxicity of lead when administered neonatally. Another study determined the neurobehavioral effects of prenatal exposure to 3,4,3',4'-tetrachlorobiphenyl in mice. Offspring of mothers given the compound on days 6-13 of gestation were assessed for neurobehavioral deficits for up to one year after birth. It was found that, when tested in adulthood, these mice demonstrated signs of neurotoxicity. The most severely affected subjects displayed a neurobehavioral syndrome consisting of intermittent sterotypic circling, head-bobbing, hyperactivity impaired forelimb grip strength, and impaired ability to traverse a wire rod. Some mice did not display the spinning syndrome but were found to be deficient in traversing a wire rod and in acquisition of an avoidance response. Thus, exposure to this polychlorinated biphenyl compound can influence neurobehavioral functioning of mice during adulthood and, in some cases, such effects can be observed in the absence of clinical signs of toxicity. Another project concerned the effects of neonatal exposure to diethylstilbestrol and testosterone on the neurobehavioral functioning of rats at various times during development. It was found that high levels of estrogen and diethylstilbestrol present during the period of sexual differentiation feminize or demasculinize the male and this effect can be observed in adulthood using appropriate sexually dimorphic neurobehavioral and morphometric measurements. Two other studies concern the long-term neurobehavioral effects of perinatal exposure of Japanese quail eggs (Coturnix coturnix japonica) to microwave irradiation or 2,4-diphenoxyacetic acid. Measures being studied are sexual and reproductive behaviors, a battery of behavioral and neurological measurements, learning and retention, and baseline performance on a multiple fixed ratio/fixed interval schedule.

NEUROCHEMISTRY

The primary goal of the Neurochemistry Workgroup is concerned with determining the biochemical bases for the effects of environmental agents on behavior and neurological function. To date the major focus of attention has been on some of the consequences of modulation of gonadal steroid production in the neonate with respect to sexual differentiation in rat brain. Monoamine oxidase levels and turnover rates are being used as one criterion for reflection of sex steroid modulation. Other criteria include estimations of serotonin and catecholamine levels and turnover rates in various parts of the brain. The results of these studies have demonstrated that steroid hormones modulate the levels of brain monoamine oxidase and that, in the 12-day old rats, there is sex specificity levels of putative neurotransmitter amines for midbrain dopamine, hypothalamic norepinephrine, and for serotonin in both corpus striatum and midbrain. Studies have been initiated concerning the effects of acrylamide on putative neurotransmitter receptor binding sites in the brain.

PERSONNEL

Additions to the Laboratory were: Research Chemist - Dr. S. C. Bondy; Visiting Fellow - Dr. S. B. Por; Technician - M. T. Riley.

OTHER ACTIVITIES

Dr. S. C. Bondy: Adjunct Associate Professor, Department of Pharmacology and the Neurobiology Program, University of North Carolina; Member, Editorial Board, Developmental Neuroscience; Member, Editorial Board, Neurotoxicology; Chairman, Session on Neurotransmitters, American Society for Neurochemistry Meeting, Charleston, South Carolina; Chairman, Session on Role of RNA and DNA, Gordon Research Conference on Macromolecules and Behavior, Wolfeboro, New Hampshire; Invited seminar entitled "The Avian Visual System as a Model for Neurobiological Studies," National Institute of Child and Human Development; Invited seminar entitled "Regulation of Cerebral Metabolism by Neurotrophic Influences," Department of Neuroscience, University of Florida Medical School, Gainesville, Florida; Invited seminar entitled "The Optic Pathway of the Chick and Its Use in Neurochemical and Behavioral Studies," North Carolina Department of Mental Health, Raleigh, North Carolina; Invited seminar entitled "The Effects of Lead and Mercury Compounds on Neurotransmitter Translocations," Winter Conference on Brain Research, Sun Valley, Idaho; Invited seminar entitled "The Bird Brain in Neurobiology," Department of Biological Sciences, Australian National University, Canberra, Australia; Invited seminar entitled "Relation of Behavior to Brain Metabolism in the Young Chick," School of Behavioral Sciences, La Trobe University, Melbourne, Australia; Invited seminar entitled

"The Preferential High Affinity Binding of the Biological Forms of Amino Acid and Hexose Stereoisometers to Bentonite," University of British Columbia, Vancouver, Canada.

Dr. P. A. Cabe: Adjunct Assistant Professor, Department of Psychology, University of North Carolina; Adjunct Assistant Professor, Department of Psychology, North Carolina State University; Member, NIH Working Group on Behavioral and Social Science Research Committee, NIEHS.

Dr. C. L. Mitchell: Adjunct Professor, Department of Pharmacology and the Neurobiology Program, University of North Carolina, lectures presented to medical and undergraduate students of the University of North Carolina; Member, Intramural Council, NIEHS; Chairperson, Animal Experimentation Committee, NIEHS; Councilor, North Carolina Society for Neuroscience; Member, Editorial Board, *Environmental Health Perspectives*; Member, Editorial Borad, *Neurotoxicology*; Member, Editorial Board, *Neurobehavioral Toxicology*; Invited seminar entitled "Neurotoxicology," Chemical Industry Institute of Toxicology; Invited seminar entitled "Testing for Neurobehavioral Toxicity," Department of Pharmacology, University of North Carolina; Invited participant, Workshop on Methods for Evaluating Effects of Toxicants on the Nervous System, San Antonio, Texas; Invited speaker, Symposium on Behavioral Toxicology, Behavioral Pharmacology Society; Participant in the US-USSR Medical Science and Public Health Agreement.

Dr. H. A. Tilson: Adjunct Associate Professor, Department of Zoology, North Carolina State University; Invited lecture entitled "Strategy for the Assessment of Neurobehavioral Consequences of Environmental Factors," Department of Zoology, North Carolina State University; Invited seminar entitled "Neurobehavioral Effects of Halogenated Hydrocarbons in Rodents," Pharmacology Department, Virginia Commonwealth University; Invited seminar entitled "A Strategy for the Development of Behavioral Tests to be Used in Neurotoxicology: An Example Using Acrylamide," Biological Sciences Seminar Series, Virginia Commonwealth University; Invited speaker at the workshop on "Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Functions," held in San Antonio, Texas; Elected member, Committee of Bench Scientists, NIEHS; Member, Standing Committee on Space, NIEHS; Member, Editorial Board, *Neurotoxicology*; Member, Editorial Board, *Neurobehavioral Toxicology*; Member, Project Advisory Group, Project to Standardize and Validate Methods and Procedures in Behavioral Teratology, sponsored by the National Center for Toxicological Research, Jefferson, Arkansas; Member, Advisory Group to the Office of Toxic Substances to assess proposals to standardize behavioral tests in neurotoxicology, Washington, D. C.; Member, Nomination Committee, Behavioral Teratology Society.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70800-03 LBNT

PERIOD COVERED
October 1, 1978 to May 1, 1979

TITLE OF PROJECT (80 characters or less)

Neurochemical Correlates of Behavioral Toxicology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. E. Wilson	Research Chemist	LBNT NIEHS
Other:	A. K. Agrawal	Visiting Fellow	LBNT NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Neurochemistry Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Several neurochemical phenomena have been investigated to determine the extent to which they might correlate with sex specific differentiation of the rat brain. Monoamine oxidase (MAO) levels were found to be sex specific in a region within the hypothalamus of the 12 day-old rat. Levels of MAO types A and B could be influenced by androgen levels in the neonate. Levels of norepinephrine, dopamine, and serotonin were sex specific in the hypothalamus, midbrain, and/or corpus striatum. Hypothalamic norepinephrine and/or midbrain serotonin in the 12 day-old rat were appropriately altered by manipulations which could be expected to influence sexual differentiation of the brain.

PROJECT DESCRIPTION

METHODS EMPLOYED: Biochemical measurements as well as surgical and dissection techniques will generally be performed according to published techniques with necessary adaptations. Behavioral testing will be performed according to designs constructed by H. Tilson, P. Cabe, and C. Mitchell.

MAJOR FINDINGS AND PROPOSED COURSE: Ontogenesis of monoamine oxidase (MAO) types A and B was similar in six regions of the brain for preweanling males and females. Highest levels of MAO were detected in the hypothalamus. Castration on day 1 resulted in diminished levels of hypothalamic MAO in the 12 day-old male. In the 12 day-old rat MAO levels in the anterior region of the hypothalamus (AHR) were greater in males than in females; however, enzyme levels in the AHR were not influenced by neonatal castration or TP administration. MAO levels in the posterior hypothalamic region (PHR) were not sex specific; however, MAO in the PHR was diminished by castration of the male neonate and that diminution could be prevented by administration of testosterone propionate (TP). This then again demonstrated that steroid hormones can modulate levels of brain MAO, however, we are not yet able to assess the possible behavioral significance of such modulation.

In the 12 day-old rat, sex specificity of levels of putative neurotransmitter amines was observed for midbrain dopamine (DA), hypothalamic norepinephrine (NE), and for 5-HT in both corpus striatum and midbrain. Results from several efforts to manipulate endogenous levels of steroid hormones indicate that endogenous levels of hypothalamic NE and of 5-HT may provide useful reflections of sex specific differentiation in the brain of the 12 day-old rat.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Alterations in local levels of and rates of turnover of biogenic amines and other putative neurotransmitter compounds are frequently associated with neurotoxic and behavioral effects of drugs and of environmental agents. Furthermore, clinical and pharmacological experience have indicated that the modulation of various transmitter metabolizing, storage, and/or transporting systems frequently lead to significant alterations in the affective state and to neurologic and psychiatric disorders.

Numerous reports have suggested that levels of monoamine oxidase in various parts of the brain or of other tissues may be of significance in signaling perturbations in regulation of local levels of biogenic amines. Levels of MAO, which vary with age and sex in brain of humans and experimental animals, are influenced by sex steroids as well as by compounds or agents of environmental significance (eg., DES, pesticides, etc.). Our efforts to determine whether and to what extent lebels of neurotransmitters and local levels of neurotransmitter metabolizing enzymes are influenced by modulation of the sex steroid levels in the neonate will be of importance in interpreting the biochemical bases of resulting behavioral alterations in treated or exposed animals.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70805-03 LBNT

PERIOD COVERED
October 1, 1977 to January 1, 1978

TITLE OF PROJECT (80 characters or less)

Neurotoxicological Effects of Polybrominated Biphenyls in Rats and Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Tilson	Head, Behavioral Toxicology Group	LBNT NIEHS
Other:	P. A. Cabe	Senior Staff Fellow	LBNT NIEHS
	J. A. Moore	Associate Director	RRP NIEHS
	C. L. Mitchell	Laboratory Chief	LBNT NIEHS

COOPERATING UNITS (if any)

Research Resources Program, NIEHS

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This is a completion report of work that was in progress at the time of the last reporting. Polybrominated biphenyls (PBB) have been recognized as an environmental hazard following the inadvertent mixture of PBB to animal feed. Rats and mice were tested after 30 days of dosing by gavage with Firemaster FF-1 (0.03-30 mg/kg), a commercial PBB-containing mixture, with hexabromobiphenyl (HBB; 0.168-16.8 mg/kg), the major PBB component, or with corn oil vehicle, and 30 days after dosing ceased. Effects observed included decreases in body weights, open-field activity, forelimb grip strength, and muscular reflexes. Loss of visual placement and hypothermia were seen in some animals. FF-1 was generally more potent than HBB. Rats were more affected than mice. Rats did not recover as well (or worsened) relative to mice during postdosing. The results suggest neurobehavioral deficits may be produced by PBB at levels lower than those which produce clinical signs of toxicity.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Male and female rats and mice were dosed with Firemaster FF-1, a commercial PBB mixture, (0.03, 3.0, and 30 mg/kg) or with 2,4,5,2',4',5'-hexabromobiphenyl (HBB; 0.168, 1.68, 16.8 mg/kg) or corn oil vehicle. HBB is the major PBB component of Firemaster FF-1; doses of FF-1 and HBB were equimolar for HBB. Doses were administered per os 5 days/week for 30 days. Testing with a battery of procedures intended to evaluate a wide range of possible deficits was then administered. A second administration of the battery occurred 30 days later, 30 days after dosing ceased. The measures included: body weight, sensorimotor reflexes (corneal reflex, pinna reflex, toe and tail pinch responses), visual placement, forelimb grip strength, open-field activity, emotionality (open-field defecations and urinations), and rectal temperature.

MAJOR FINDINGS AND PROPOSED COURSE: Relative to controls, dosed animals showed decreased body weights, depressed open-field activity, lower forelimb grip strength, and depressed reflexes. An apparent loss of visual placement and the occurrence of hypothermia were noted in some animals. Animals dosed with FF-1 were generally more affected than those dosed with HBB, and rats seemed to be more affected than mice. Mice showed more postdosing recovery than rats. Rats, in fact, appeared to have deteriorated somewhat in the post-dosing period. No obvious overt signs of toxicity were observed. Thus, PBB may produce neurobehavioral effects at doses lower than those which yield clinical signs of toxicosis.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This work is related to the program of the Institute in that it provided basic research into an animal model of human environmental disease. It is significant for biomedical research in that it was demonstrated that the animal model could provide 'early warning' in the sense that the neurobehavioral tests differentiated dosed from undosed animals, in two species, before signs of clinical toxicosis could be seen.

PUBLICATIONS

Tilson, H. A., Cabe, P. A., and Mitchell, C. L.: Behavioral and neurological toxicity of polybrominated biphenyls in rats and mice. Environ. Health Perspect. 23: 257-263, 1978.

Tilson, H. A. and Cabe, P. A.: Studies on the neurobehavioral effects of polybrominated biphenyls in rats. Ann. N. Y. Acad. Sci. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 90000-02 LBNT

PERIOD COVERED
January 1, 1978 to July 1, 1978

TITLE OF PROJECT (80 characters or less)

Toxicity of Selenium, Alone and in Combination with Silver or Arsenic, in Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. A. Cabe	Senior Staff Fellow	LBNT NIEHS
Other:	N. Carmichael	Visiting Fellow	LOFT NIEHS
	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT NIEHS

COOPERATING UNITS (if any)

Laboratory of Organ Function and Toxicology, NIEHS

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The present work looked for a detoxification interaction between selenium and a relatively nontoxic metal, silver, in rats. Since metals, including selenium, have been reported to have neurotoxic effects, a limited set of neurobehavioral measures was also taken, concentrating on motor function measures. Body weight decreases occurred in the groups dosed with selenium, though gross food consumption was elevated. Water consumption was decreased in all dosed groups. The pattern of results does not allow an inference of unequivocal neurotoxicity for selenium under the conditions employed. The fact that body weight differences occurred in the selenium alone but not the silver alone or silver/selenium group argues for a protective effect of silver against some general health effects of selenium. A parallel experiment assessed the interaction of arsenic and selenium on general health and motor functioning measures. Arsenic and selenium in combination killed 7/10 animals. Consumption measures showed consistent effects but motor function was not reliably affected.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: In these two experiments, rats were dosed via drinking water, in the Ag/Se experiment with 1000 ppm Ag as silver nitrate, 10 ppm Se as sodium selenate, or a solution containing both 10 ppm Se and 1000 ppm Ag over 12 weeks and in the As/Se experiment with 50 ppm As, 10 ppm Se, or a solution containing 50 ppm As and 10 ppm Se over 18 weeks. Body weights and food and water consumption were taken weekly. Neurobehavioral assessments occurred biweekly in the Ag/Se experiment and triweekly in the As/Se study. Motor functioning was analyzed in tests of spontaneous activity, forelimb grip strength, hindlimb extensor response, and inclined screen coordination.

MAJOR FINDINGS AND PROPOSED COURSE: These experiments have been completed. Body weights for the selenium alone group were depressed from the third week of dosing on, although food consumption (g food/g body weight/day) was elevated for those animals. Food consumption for the silver/selenium group was also typically elevated, though not as markedly. Water consumption (ml water/g body weight/day) was decreased consistently for all dosed animals. That body weights for the silver/selenium and silver alone groups never differed from controls suggests that silver has a protective effect against selenium toxicity. Seven of the ten animals treated with arsenic and selenium died over the course of the experiment. Consumption measures were again the most clearly affected. No consistent pattern of motor function effects could be seen. The motor function tests showed no consistent pattern, although when (transient) differences from controls occurred they were more likely to be in the selenium alone group than any other. It would be difficult to conclude that selenium produced a neurotoxic effect under the test conditions used. Further research, however, with a more extensive battery of tests appears to be warranted, especially including tests of sensory function. A manuscript describing this work is in preparation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Selenium in excess is known to have effects on human health. Its putative detoxifying effect with other metals, especially given its demonstrated nutritional essentiality, suggests a possible therapeutic mechanism in metal intoxication. The possibility of a role for selenium in human metal toxicity therapy places this research within the scope of the Institute's program.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 90001-02 LBNT

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Effects of Acrylamide on Food and Water Consumption and Spontaneous Activity of Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Squibb	Staff Fellow	LBNT NIEHS
Other:	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT NIEHS
	S. C. Bondy	Head, Neurochemistry Workgroup	LBNT NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of these studies is to determine the effects of chronic exposure to acrylamide on patterns of diurnal and noctural food and water consumption, as well as the spontaneous locomotor activity of rats in domiciliary cages. Attempts will be made to measure changes from expected baselines in each behavioral variable as a function of dose, length of exposure to acrylamide, age and sex of rat. Behavioral dysfunction and interaction of organismic variables will be associated with physiological and neurochemical measures.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Rats of the Fisher-344 strain exposed to acrylamide *in utero* or as adults will be individually housed in a specially constructed domiciliary battery. The temporal patterning food and water consumption bouts and home cage crossing activity will be monitored on-line by a laboratory computer.

In addition, the effects of acrylamide on the turnover and disposition of select brain neurotransmitters, such as dopamine and norepinephrine, in various regions of the central nervous system will be determinated.

MAJOR FINDINGS AND PROPOSED COURSE: These experiments are in progress.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies will attempt to identify subtle behavioral changes in the patterning of consummatory and spontaneous motility patterns of rats exposed to low levels of acrylamide and to correlate those changes with physiological and neurochemical alterations. The results of these studies may lead to the identification of highly sensitive bio-behavioral assays for the assessment of neurotoxic agents.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 90002-02 LBNT

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of Diet and Food Restriction on Acrylamide Toxicity in Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Squibb	Staff Fellow	LBNT NIEHS
Other:	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of these experiments is to determine the effects of two commonly used diets and food restriction on the neurobehavioral toxicity of acrylamide in rats. Changes in consummatory and neurobehavioral measures as a function of cumulative dose and duration of exposure to acrylamide, diet, and food restriction will be studied.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: One hundred male rats of the Charles River CD strain, weighing 170-190 g served as subjects for the first experiment. They were individually housed in stainless steel suspension cages. Half the subjects (n=50) were fed *ad libitum* an open formula cereal based diet, NIH 31; the rest were given Purina 5755, an open formula diet with purified ingredients. The subjects of each diet group were divided into five groups (n=10) and dosed once by gavage with 50, 100, 150, and 200 mg/kg of acrylamide. The number of lethalities at 24 hrs and 168 hrs postdose were counted. Body weights, food and water consumption, and performance on an inclined screen were measured at these times. A second experiment was conducted with 0, 100, 200, 250, and 300 mg/kg. The purpose of this study was to insure sufficient number of lethalities in each of the diet groups to estimate the LD50 of acrylamide with 90% confidence intervals by the Spearman-Karber method.

MAJOR FINDINGS AND PROPOSED COURSE: Estimates of the LD50 with 95% confidence limits showed that the lethal effects of orally administered acrylamide was greater when subjects were maintained on the Purina 5755 open formula purified diet. At 24 hrs postdose, the estimated LD50 was 180 mg/kg (136.9-223 confidence limit). With the NIH 31 cereal diet, the number of lethalities at 24 hrs postdose were not sufficient to estimate the LD50; at 72 hrs postdose, however, it was estimated to be at 232.5 mg/kg (211.5-253.5 confidence limit).

Reduced body weights 24 hrs postdose were significant according to the amount of acrylamide given. The interaction term between treatments and diets was not significant. By 168 hrs, postdose, body weight recoveries were almost complete in the high dose groups. Dose-related aphagia and adypsia were evident by 24 hrs postdose, the interaction between treatments and diets was not significant. At 168 hrs, the food consumption of all treatment groups recovered to control values. At the high dose levels, however, polydypsia was highly significant.

Behavioral performance rating scores in an inclined screen test revealed that the severity of acrylamide toxicity, after 24 hrs, was significantly greater in the purified diet fed rats. At 168 hrs, the results of the inclined screen test showed that functional recovery to control values had occurred.

An experiment to study the effects of diet with repeated exposures to subacute doses of orally administered acrylamide is planned. Additional behavioral tests will include spontaneous motor activity in an automex apparatus, fore- and hindgrip strength, and startle responsitivity to auditory and air puff stimuli. Body weight performance and measures for food and water consumption will also be taken on a weekly basis before, during, and after the period of exposure to acrylamide.

Future studies on diet interaction will examine the uptake distribution and metabolism of acrylamide. Select neurochemical mechanisms such as dopamine receptor binding activity may also be examined. Whether the neurobehavioral toxic effects of acrylamide could be potentiated or attenuated by nutritional deficiencies or supplementations, eg., proteins and vitamins will also be considered.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The effects of diet and nutrition on the manifestation of functional neurobehavioral toxicity has not been studied systematically, although the nutritional status of the organism has obvious importance. Results from these studies may provide insight into the possible role of nutritional factors on the mechanism of action of acrylamide toxicity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 90003-02 LBNT

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of Developmental Exposure to Polychlorinated Biphenyls on Neurobehavioral Functioning of Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT NIEHS
Other:	G. W. Lucier	Acting Chief	LOFT NIEHS

COOPERATING UNITS (if any)
Laboratory of Organ Function and Toxicology, NIEHS
Endocrine Labs of Madison, Madison, Wisconsin

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.1	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of these experiments is to determine the neurobehavioral effects of prenatal exposure to 3,4,3',4'-tetrachlorobiphenyl (TCBs) in mice. Offspring of mothers given TCBs on days 6-13 of gestation are being assessed for neurobehavioral deficits for up to 1 year after birth.

PROJECT DESCRIPTION

METHODS EMPLOYED: Pregnant CD-1 mice are given 3,4,3',4'-tetrachlorobiphenyl (TCB) by gavage on days 6-13 of gestation. The TCB-exposed offspring are assessed for the presence or absence of rapid circular movements or "spinning" at a time prior to weaning. At 21 days of age, spinning and nonspinning mice are selected with control mice for evaluation in a longitudinal behavioral study. The measures include: exploratory activity, rectal temperature, forelimb grip strength, visual placement, ability to balance on a wire, acquisition of one-way avoidance response, and body weights. Tests are conducted at one and six months of age. In addition, diurnal activity of mice housed in groups of four is assessed over a 4-5 day period following each neurobehavioral assessment.

MAJOR FINDINGS AND PROPOSED COURSE: When tested at adulthood, albino mice exposed *in utero* to 3,4,3',4'-tetrachlorobiphenyl (TCB) demonstrated signs of neurotoxicity. The most severely affected subjects (TCB-Spinners) displayed a neurobehavioral syndrome consisting of intermittent stereotypic circling, head-bobbing, and hyperactivity, TCB-Spinners were found to be markedly hyperactive during the dark phase of the diurnal phase and showed impaired forelimb grip strength, ability to traverse a wire rod, visual placement responding, and acquisition of one-way avoidance. Some mice did not display the spinning syndrome (TCB-Nonspinners), but were found to be deficient in traversing a wire rod and avoidance acquisition. None of the TCB-exposed mice were found to have depressed neuromuscular reflexes in response to a variety of stimuli. Tissue distribution studies demonstrated that TCB levels were not detectable in adult mice following prenatal exposures. The results of these experiments demonstrate that prenatal exposure to TCB can influence neurobehavioral functioning of mice during adulthood and, in some cases, such effects can be observed in the absence of clinical signs of toxicity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The behavioral teratogenic potential of TCBs has not been assessed systematically. The experiments described above will provide a long range evaluation of perinatal exposure to PCBs on neurobehavioral functioning. Some insight into the mechanism by which perinatally administered TCBs affects the nervous system may also be attained.

PUBLICATIONS

Tilson, H. A., Davis, G. J., McLachlan, J. A., and Lucier, G. W.: The effects of polychlorinated biphenyls given prenatally on the neurobehavioral development of mice. *Environ. Res.* (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 90004-02 LBNT

PERIOD COVERED

December 15, 1977 to July 1, 1978

TITLE OF PROJECT (80 characters or less)

Neurobehavioral Assessment of Rats Given Carbon Disulfide

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT NIEHS
Other:	E. W. Van Stee	Head, Inhalation Toxicology Section	EBB NIEHS
	P. A. Cabe	Senior Staff Fellow	LBNT NIEHS

COOPERATING UNITS (if any)

Duke University
Inhalation Toxicology Section, Environmental Biology Branch, NIEHS

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Behavioral Toxicology Workgroup

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to assess changes in neurobehavioral functioning of rats at various times during and after exposure to carbon disulfide by inhalation. Changes in the functioning of catecholaminergic receptors will also be determined in animals dosed with carbon disulfide.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Male, albino rats of the Fisher strain were exposed to 2 mg/l of carbon disulfide (CS_2) by inhalation four hours per day, five days per week for six consecutive weeks. At weeks three and six during dosing and at one and three weeks after cessation of dosing, rats were assessed for neurobehavioral functioning using the following measures: exploratory motor activity, startle responsiveness, forelimb grip strength, and hind limb extensor response. Body weights were taken weekly during the course of the experiment.

Some rats were tested after six weeks of dosing and three weeks after cessation of dosing for changes in the sensitivity of catecholaminergic functioning. Spontaneous motor activity, startle responsiveness, and stereotypic motor responses were studied following systemic administration of d-amphetamine (6 mg/kg).

MAJOR FINDINGS AND PROPOSED COURSE: Male, albino rats of the Fisher strain were exposed to 2 mg carbon disulfide/l of air for four hours per day, five days per week for six weeks. Neurobehavioral effects were not observed after three weeks of dosing, but hindlimb extensor responses and performance on the inclined screen (motor coordination) were impaired after six weeks of exposure. Forelimb grip strength and exploratory locomotor activity were not affected. Three weeks after cessation of dosing, recovery of function was observed.

Rats exposed to CS_2 for six weeks were found to be stimulated less than air ventilated controls by 3 mg/kg of d-amphetamine. In addition, d-amphetamine-induced augmentation of an acoustic startle response was attenuated in CS_2-exposed rats. These data suggest that repeated exposure to CS_2 affects the availability of brain norepinephrine for release. Enhanced responsiveness to the stereotypic effects of a high dose of d-amphetamine (6 mg/kg) suggested changes in functioning of dopaminergic systems. Three weeks after cessation of dosing, there was no difference between groups in their response to d-amphetamine.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies will aid in the elucidation of the mechanism of action of CS_2, as well as provide information as to appropriate tests to be used in the study of changes in brain functioning during repeated exposure to neurotoxins.

PUBLICATIONS

Tilson, H. A., Cabe, P. A., Ellinwood, E. H., and Gonzalez, L. P.: Effects of carbon disulfide on motor function and responsiveness to d-amphetamine in rats. Neurobehav. Toxicol. 1: 57-63, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 90005-02 LBNT

PERIOD COVERED
October 1, 1978 to January 31, 1979

TITLE OF PROJECT (80 characters or less)
Effects of Equal Doses of Firemaster and 2,4,5,2',4',5'-Hexabromobiphenyl in Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT NIEHS
Other:	P. A. Cabe	Senior Staff Fellow	LBNT NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The neurobehavioral effects of 2,4,5,2',4',5'-hexabromobiphenyl (HBB) and Firemaster FF-1, a commercial mixture of polybrominated biphenyls (PBB), of which HBB is the major component, have been shown to be different when given in amounts equating HBB content. The present study repeats and extends observations of neurobehavioral effects of equal doses of HBB or FF-1. Rats were trained to press a lever in standard operant conditioning chambers to avoid an unsignaled presentation of electric footshock and to escape shock when it is presented (unsignaled continuous avoidance) until a stable baseline of responding is achieved. Dosing (10 mg/kg of FF-1, 10 mg/kg of HBB, or corn oil vehicle) was started. Effects on performance of the avoidance schedule were evaluated.

PROJECT DESCRIPTION

METHODS EMPLOYED: Three groups of rats were initially trained in standard operant conditioning chambers to press a lever in daily (5/week) sessions to avoid electric shock or to terminate it (escape) when it occurred. When responding stabilized, dosing by gavage with 10 mg/kg Firemaster, 10 mg/kg HBB, or corn oil vehicle was started. At the end of the 8 week dosing period, rats were given 7.5-30 mg/kg of phenobarbital or 0.25-2 mg/kg of d-amphetamine, i.p., prior to behavioral sessions.

MAJOR RESULTS AND PROPOSED COURSE: HBB or Firemaster had no effect on the rate of responding, shocks delivered or distribution of inter-response times after eight weeks of dosing. However, alterations in the responsiveness of the rats to the behavioral depressant effects of phenobarbital. Rats treated with HBB or FF-1 were less sensitive to phenobarbital than controls. Difference between PBB-exposed rats and controls with regard to responsiveness to d-amphetamine were not observed. The attenuation of the effects of phenobarbital by PBBs may be explained by the fact that PBBs are potent inducers of hepatic metabolic enzymes. Although the operant behavioral procedure used in this study was not able to detect subtle changes in neurobehavioral functioning following repeated dosing with FF-1 and HBB, the use of a pharmacological challenge unmasked functional changes in the responsiveness of PBB-exposed rats to phenobarbital.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Interactive effects of chemical substances, especially in commercial-grade preparations, will be a continuing problem in environmental health. PBB toxicity is of immediate concern because it has affected relatively large human populations. Evaluation of substances such as PBB, which clearly affect human health, is specifically within the mandate of the Institute.

PUBLICATIONS

Tilson, H. A. and Cabe, P. A.: Studies on the neurobehavioral effects of polybrominated biphenyls in rats. Ann. N. Y. Acad. Sci. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 90006-02 LBNT

PERIOD COVERED
October 1, 1978 to January 1, 1979

TITLE OF PROJECT (80 characters or less)
Neurobehavioral Toxicity of Developmental Exposure to 2,4-Diphenoxyacetic Acid (2,4-D) in Japanese Quail

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT NIEHS

COOPERATING UNITS (if any)
Zoology Department, North Carolina State University
Poultry Science Department North Carolina State University

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In spite of their wide use, the behavioral and neurological toxicity of herbicides, such as 2,4-diphenoxyacetic acid (2,4-D), have not been well studied. The purpose of the present study is to investigate the effects of exposing Japanese quail (Corturnix corturnix japonica) eggs to varying concentrations of commercially available and technical grade 2,4-D on the development of neurobehavioral functioning.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Fertilized Japanese quail eggs will be exposed to varying concentrations of 2,4-D, Weedar-64 and a vehicle of inert substances. After incubation, the effects of hatching time, percentage hatched, and growth rates of quail will be assessed. At six to eight weeks of age, the mating behavior and reproductive capabilities of male and female quail of each group will be assessed. At six months of age and every six months thereafter, spontaneous motor activity, flight thrust, and startle responsiveness to a visual stimulus will be evaluated. Acquisition and retention of a shuttle box avoidance response will also be assessed. Body weights will be recorded weekly.

At six to eight weeks of age, other groups of quail treated in the egg with 2,4-D will be food-deprived to 80-85% of their free-feeding body weight and trained to peck a key for food reinforcement. After baseline performance has been established, the quail will be trained to respond on a FR FI schedule of food reinforcement. Acquisition of operant behavior and baseline performance parameters will be evaluated for indications of neurotoxicity.

MAJOR FINDINGS AND PROPOSED COURSE: This project has been terminated. The project demonstrated the usefulness of several procedures (eg., spontaneous activity, sexual functioning) for the assessment of neurobehavioral functioning in Coturnix quail but pointed out the need for further normative development of other methods (eg., shuttle escape-avoidance, operant conditioning). While some treatment group differences occurred, the results of the study cannot be interpreted as indicating a consistent chemical effect, primarily due to uncertainties about the administration procedure attempted. Further study, however, may be warranted, pending additional procedural development and evaluation of alternative dosing regimens.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Herbicides such as 2,4-D are often regarded as relatively safe environmental agents because of the manner of application in relation to the time and method of crop harvest and the rapid biodegradability. The effects of exposure to herbicides and their metabolites on developing organisms are relatively unknown and are in need of systematic scientific evaluation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 90007-02 LBNT

PERIOD COVERED
October 1, 1978 to April 1, 1979

TITLE OF PROJECT (80 characters or less)

Neurobehavioral Toxicity of Neonatal Exposure of Rats to Diethylstilbestrol

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT NIEHS
Other:	C. Lamartiniere	Senior Staff Fellow	LOFT NIEHS
	O. Meyer	Visiting Scientist	LET NIEHS

COOPERATING UNITS (if any)
Laboratory of Organ Toxicology and Function, NIEHS
Laboratory of Environmental Toxicology, NIEHS

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this research is to determine the effects of chemicals on sexual differentiation during development as measured by sexually dimorphic morphometric and neurobehavioral parameters during adulthood. Intact and castrated rat pups are dosed neonatally with hormonal substances such as diethylstilbestrol, estrogen and testosterone and body weight, forelimb grip strength, and emergence behavior studied at 61 days of age.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: At 61 days of age, the body weights, forelimb grip strength, and latency to emerge into a novel environment are measured. At 63 days of age, the animals are sacrificed. The testes of intact rats are assessed histologically for the presence of spermatogenesis.

MAJOR FINDINGS AND PROPOSED COURSE: Intact or neonatally castrated male albino rats of the Sprague-Dawley strain were given s.c. injections of propylene glycol (PG), diethylstilbestrol (DES), estradiol-17β (E_2), or testosterone propionate (TP) on days 2, 4, and 6 postpartum. Intact females given PG neonatally were tested for comparative purposes. The neonatal administration of DES and E_2 to intact pups attenuated or blocked the course of sexual differentiation. Gonadal development of the E_2- and DES-treated animals was affected significantly, and the adult body weights, grip strength scores, and emergence latencies of these animals were not different from intact females given PG. Except for a delay for gonadal development, neonatal administration of TP had no significant neurobehavioral effects. The adult body weights, grip scores, and emergence latencies of male pups castrated at birth were similar to those of female rats and their effects were not altered by neonatal administration of DES or E_2. Neonatal administration of TP appeared to block the effect of castration on body weights and grip strength scores, but did not alter emergence latencies. Intact males given DES or E_2 and castrates given PG, DES, or E_2 did not differ significantly from intact females given PG neonatal. These data indicate that high levels of E_2 and DES present during the period of sexual differentiation feminize or demasculinize the male and this effect can be observed in adulthood using appropriate sexually dimorphic neurobehavioral and morphometric measurements.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There is a need to understand more fully the effects of perinatal exposure to diethylstilbestrol and other hormonal agents on neurobehavioral functions other than reproductive and sexual behaviors. The ability to detect other, more subtle behavioral teratogenic effects may prove useful in determining the presence of previously unknown or poorly described psychological and neurobehavioral deficits. In addition, such studies may prove useful in elucidating the mechanism and site of action of such agents.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 90008-02 LBNT

PERIOD COVERED
October 1, 1978 to January 15, 1979

TITLE OF PROJECT (80 characters or less)

Acute and Chronic Neurobehavioral Toxicity of Acrylamide in Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT NIEHS
Other:	P. A. Cabe	Senior Staff Fellow	LBNT NIEHS

COOPERATING UNITS (if any)

Albert Einstein College of Medicine of Yeshiva University, Bronx, New York

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of acrylamide on the neurobehavioral functioning of rats were studied in order to facilitate the validation of a primary test screen. Male Fisher rats were studied under three experimental conditions: (1) 12 and 168 hours after a single dose (50-200 mg/kg, p.o.), (2) weekly during a course of repeated dosing, five times weekly for four weeks (10-20 mg/kg, p.o.) and for two weeks after cessation of dosing, and (3) every third week during a course of repeated dosing, three times weekly for 13 weeks (10-20 mg/kg, p.o.) and at one and five weeks postdosing. The sensitivity of various tests for motor functioning were examined.

PROJECT DESCRIPTION

METHODS EMPLOYED: Subjects have been Fisher strain albino rats, typically 70 days of age at the beginning of the experiment. Rats are housed individually with food and water freely available. The measures of motor functioning that were obtained include: hindlimb extensor response, forelimb grip strength, and general motor activity in a novel environment.

MAJOR FINDINGS AND PROPOSED COURSE:

(1) Lethal dose. A lethal dose (LD50) of 251 mg/kg was found at 12 hours and 175 mg/kg at 168 hours.

(2) Acute dosing. Caudal motor dysfunction was observed in the hindlimb extensor response and inclined screen tests 12 hours after a single dose of 50-200 mg/kg. Hindlimb function recovered completely by 168 hours. No effect on forelimb strength was observed.

(3) Short-term repeated dosing. Repeated 10-20 mg/kg doses over four weeks produced hindlimb deficits at cumulative doses of 50-100 mg/kg. No forelimb dysfunction appeared. General motor activity was decreased after four weeks of dosing. Recovery of function was incomplete at two weeks after cessation of dosing.

(4) Longer term repeated dosing. Rats receiving 20 mg/kg had significantly lower body weights at 4-13 weeks of dosing. Significant decreases in hindlimb function were observed during 7-13 weeks of dosing, while motor activity was decreased at 10-13 weeks. Forelimb grip strength was not affected during the dosing period. In rats receiving 10 mg/kg, there were no effects during the dosing period.

At the end of the 13 weeks of dosing, signs of early fiber degeneration were observed in the peripheral nerves of all animals receiving 10 mg/kg of acrylamide, while the rats in the high dose group were found to have signs of moderate to severe acrylamide neurotoxicity. One week after cessation of dosing, fore- and hindlimb scores, spontaneous motor activity, and body weights were significantly decreased in the high dose rats. Five weeks after cessation of dosing, the acrylamide-treated rats did not differ from controls on neurobehavioral measures. Neuropathological examination showed signs of regenerating and remyelinating fibers in the animals previously exposed to acrylamide.

The neurobehavioral screening procedures used in these experiments show both sensitivity and specificity to the neurotoxic effects of acrylamide. These and other studies in our laboratory may prove beneficial in the development of a battery of tests to be used to assess the neurological and behavioral toxicity of many types of environmental factors.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The validation of neurobehavioral test methods is a crucial step in the development of neurotoxicological predictive capabilities. The studies described here provide an invaluable data base for further methods development, using a substance which in itself is a known environmental contaminant. The studies described are congruent with the Institute's mission to discover methods for the detection and prediction of environmental health hazards.

PUBLICATIONS

Cabe, P. A. and Tilson, H. A.: Hindlimb extensor response: A method for assessing motor dysfunction in rats. Pharmacol. Biochem. Behav. 9: 113-136, 1978.

Tilson, H. A. and Cabe, P. A.: Assessment of chemically-induced changes in the neuromuscular function of rats using a new recording grip meter. Life Sci. 23: 1365-1370, 1978.

Tilson, H. A. and Cabe, P. A.: The effects of acrylamide given acutely or in repeated doses on fore- and hindlimb function of rats. Toxicol. Appl. Pharmacol. 47: 253-260, 1979.

Tilson, H. A. and Cabe, P. A.: A strategy for the assessment of neurobehavioral consequences of environmental factors. Environ. Hlth. Perspec. 26: 287-299, 1978.

Tilson, H. A., Spencer, P. S., and Cabe, P. A.: Acrylamide neurotoxicity in rats: A correlated neurobehavioral and pathological study. Neurotoxicol. 1: 89-104, 1979.

Tilson, H. A., Cabe, P. A., and Burne, T. B.: Procedures to be Used in the Routine Assessment of Neurotoxicity in Rodents: Effects of Acrylamide. In Spencer, P. S. and Schaumburg, H. H. (Eds.): Experimental and Clinical Neurotoxicology: A Textbook of Environmental Neurobiology. Baltimore, Maryland, Williams and Wilkins (in press).

Tilson, H. A., Mitchell, C. L., and Cabe, P. A.: Screening for neurobehavioral toxicity: The need for and examples of validation of testing procedures. Neurobehav. Toxicol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 90009-02 LBNT

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Neurobehavioral Toxicity of Developmental Exposure of Japanese Quail to Microwave Irradiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. A. Cabe	Senior Staff Fellow	LBNT NIEHS
Other:	D. I. McRee	Research Physicist	LEB NIEHS
	C. L. Mitchell	Laboratory Chief	LBNT NIEHS
	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT NIEHS

COOPERATING UNITS (if any)
Laboratory of Environmental Biophysics, NIEHS
Zoology Department, North Carolina State University
Poultry Science Department, North Carolina State University

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long-term neurobehavioral effects of perinatal exposure of Japanese quail eggs (*Coturnix coturnix japonica*) to 2450 MHz CW microwave irradiation at a power density of 5 mW/cm^2 for the first 12 days of incubation will be assessed in this experiment. Measures include sexual and reproductive behaviors, a battery of behavioral and neurological measurements, learning and retention, and baseline performance on an operant schedule. Work already completed tentatively suggests differences on learning measures.

PROJECT DESCRIPTION

METHODS EMPLOYED: Fertilized Japanese quail eggs will be exposed to 2450 MHz CW microwave irradiation at a power density of 5 mW/cm^2 for the first 12 days of development. The effects of irradiation on hatchability and number of deformities of exposed and control eggs will be observed.

At six to eight weeks, the mating behavior and reproductive capabilities of male and female quail from each group will be assessed. Measures of neurobehavioral functioning (startle responsiveness, flight thrust, spontaneous motor activity) will be obtained at six months of age and every six months thereafter for a period of up to two years. The ability to acquire a two-way active avoidance response will be tested at six months of age and retention testing will occur every six months. General health indicators (body weight, food consumption) will also be assessed.

A second group of exposed and control male quail will be food-deprived to 80-85% of their free-feeding body weights at 6-8 weeks of age and trained to peck a lighted key for food reinforcement. Following measures of acquisition abilities, the quail will be trained to respond on a mult FR FI schedule. Baseline parameters regarding responding under discriminative control and patterning of responding under various schedule components will be assessed.

MAJOR FINDINGS AND PROPOSED COURSE: This project is underway. Results from this study will be used to determine the usefulness of the quail as a subject for behavioral teratological research and for the study of physical factors such as irradiation. This experiment will also be the basis for future investigations concerning the effects of microwave irradiation on neurobehavioral development. Pilot work completed to date suggests that shuttle escape-avoidance performance and acquisition of an autoshaped key-peck response may discriminate between exposed and control animals. Body weights have not been seen to be affected and spontaneous activity has shown no difference. Operant baseline performance under a random-interval schedule of reinforcement is currently under test.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Microwave-generating devices are employed in a variety of military, industrial, and civilian applications. However, the consequences of prenatal exposure to microwave irradiation on neurobehavioral functioning have not been studied systematically, in spite of the known sensitivity of developing organisms to many types of chemical and physical insults.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 90010-02 LBNT

PERIOD COVERED
October 1, 1978 to April 13, 1979

TITLE OF PROJECT (80 characters or less)

Neurobehavioral Effects of Chronic Polybrominated Biphenyls in Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT NIEHS
Other:	P. A. Cabe	Senior Staff Fellow	LBNT NIEHS
	J. A. Moore	Associate Director	RRP NIEHS

COOPERATING UNITS (if any)

Research Resources Program, NIEHS

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.4	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Polybrominated biphenyls (PBB) have been reported to produce clinical signs of neurological toxicity in humans and neurobehavioral deficits in rats in relatively short exposures. The present work was intended to assess neurobehavioral consequences of chronic (six month) exposure to a commercial mixture of PBB (Firemaster FF-1). Rats received 0.3, 1.0, or 3.0 mg/kg body weight of FF-1 or corn oil vehicle. Both male and females rats were tested in a battery designed to screen for neurobehavioral dysfunction. Tests included: body weight, motor activity, forelimb grip strength, hindlimb strength, startle to air puff, and rectal temperature. Shock escape latencies and acquisition of shock avoidance were tested at the end of six months of dosing.

PROJECT DESCRIPTION

METHODS EMPLOYED: Rats were dosed orally on a five day/week schedule for 180 days, at 0.3, 1.0, or 3.0 mg/kg. Controls were dosed with corn oil vehicle. The rats were administered the following tests: spontaneous motor activity (automated monitors), forelimb grip strength, hindlimb extensor response, and startle. Latency to avoid signaled shock and acquisition of shock avoidance were tested.

MAJOR FINDINGS AND PROPOSED COURSE: The effects of repeated dosing with Firemaster BP-6 (FF-1), a mixture of polybrominated biphenyls (PBBs), the neurobehavioral functioning of Fisher, albino rats were studied. After total doses of 390-1300 mg/kg over a six month period, there were significant decreases in locomotor activity, forelimb grip strength, and hindlimb extensor responding. PBBs did not result in hyperreflexia or irritability but instead suppressed startle responsiveness to an air puff stimulus.

The results of these experiments indicate that repeated exposure to PBBs produce signs of behavioral suppression and muscular weakness. Other indications of neurotoxicity, such as irritability and impaired learning ability were not observed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Chronic effects of environmental toxins are of continuing concern in environmental medicine. The controlled evaluation of effects of PBB is a current problem in human health, in that an affected population and a population at risk exist; the Institute's programs are aimed specifically at dealing with such environmental health issues.

PUBLICATIONS

Tilson, H. A. and Cabe, P. A.: Studies on the neurobehavioral effects of polybrominated biphenyls in rats. Ann. N. Y. Acad. Sci. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 90011-01 LBNT

PERIOD COVERED
April 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Behavioral and Morphological Effects of Triethyl Tin in Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Squibb	Staff Fellow	LBNT NIEHS
Other:	N. E. Carmichael	Visiting Fellow	LOFT NIEHS

COOPERATING UNITS (if any)

Laboratory of Organ Function and Toxicology

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of these studies is to correlate the neurobehavioral effects of repeated exposure to triethyl tin bromide with the neuromorphology of white matter tracts in the cerebellum and medulla of rats. Body weight losses with changes in food and water consumption will be compared to the advent of CNS and PNS functional deficits as measured by motor activity, startle responsiveness, and fore-hindgrip strength tests.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Forty male rats of the Fisher-344 strain will be housed individually in stainless steel suspension cages with food (NIH 31) and water provided *ad libitum*. They will be dosed p.o. twice a week, for three weeks with doses of 1.0, 2.0, and 3.0 mg/kg triethyl tin bromide. Once a week individual body weights and food and water consumption will be measured. Spontaneous motor activity, startle responsiveness to both auditory and air puff stimuli, and fore-hindgrip strength tests will also be conducted. After dosing, half the subjects of each dose group will be sacrificed for histopathological examination, while the rest will be allowed a 30-day period for recovery. Measures of body weights and food and water consumption, as well as performance on behavioral tests, will be taken. Histopathology of brain tissue will take place immediately thereafter.

MAJOR FINDINGS AND PROPOSED COURSE: Preliminary work to standardize histological procedures has shown that repeated doses of 1.0, 2.0, and 3.0 mg/kg triethyl tin bromide produces loss of body weight. There was evidence of severe myelin edema after a cumulative toxic dose of 12.0 mg/kg in white matter tracts in the cerebellum and medulla. Ongoing experiments will attempt to systematically determine the advent of behavioral deficits, if present, with body weight and consummatory changes, and with the neuropathology of chronic tin toxicity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies will attempt to correlate the sensitivity of select functional tests with the pathology of tin toxicity and with changes in naturally occurring behavioral responses such as spontaneous motor activity and consummatory behaviors. The results of these studies may lead to the identification and application of highly sensitive biobehavioral assays to biomonitor the chronic effects of environmental neurotoxic agents such as the heavy metals.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 90012-01 LBNT

PERIOD COVERED

January 1, 1979 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of Developmental Exposure to Monosodium Glutamate on the Neurobehavioral Development of Rats.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT NIEHS
Other:	C. Lamartiniere	Senior Staff Fellow	LOFT NIEHS
	O. Meyer	Visiting Scientist	LET NIEHS

COOPERATING UNITS (if any)

Laboratory of Organ Function and Toxicology, NIEHS
Laboratory of Environmental Toxicology, NIEHS

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Behavioral Toxicology Workgroup

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.2	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Sprague-Dawley rat pups of both sexes were given monosodium glutamate subcutaneously on days 1-5 postnatally. The neurobehavioral development of the rats was assessed in a battery of tests at various times pre- and post weaning.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Timed pregnant Sprague-Dawley rats were allowed to deliver and the offspring given monosodium glutamate (MSG) s.c. daily from days 1-5 postpartum. Doses were 2.0-3.5 mg/g body weight. Controls received injections of 0.9% isotonic salene. The rats were given a battery of tests design to measure a wide range of sensory and motor functions. Tests were administered prior to weaning and at 30-40, 60-70, and 100-110 days of age.

MAJOR FINDINGS AND PROPOSED COURSE: The experiment is still in progress.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The study of how exposure to environmental agents during the period of sexual differentiation affects neurobehavioral functioning may yield information concerning the neurohumoral and neuroendocrine mechanisms underlying behavioral teratogenic effects.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 90013-01 LBNT

PERIOD COVERED
March 28, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Neurobehavioral Effects of Repeated Exposure to Kepone in Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT NIEHS
Other:	J. A. Moore	Associate Director	RRP NIEHS
	S. C. Bondy	Head, Neurochemistry Workgroup	LBNT NIEHS

COOPERATING UNITS (if any)
Research Resources Program, NIEHS

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.2	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Male and female Fisher rats were exposed to 10-30 ppm Kepone via the diet for 51 days. At the end of dosing, they were assessed for neurobehavioral dysfunction using a battery of tests. Approximately 28 days after cessation of dosing, the rats were assessed for recovery of function. Binding characteristics of brain catecholamine systems will be assessed.

PROJECT DESCRIPTION

METHODS EMPLOYED: Male and female albino rats of the F-344 strain approximately 6-8 weeks old at the beginning of the study were used as subjects. The animals were randomly assigned to receive either 0, 10, or 30 ppm of kepone in the diet for a period of 51 days. There were eight rats of each sex per each treatment group and 12 rats in the control groups. At the end of the 51 day dosing period, the rats were given a battery of neurobehavioral tests during days 56-58 of the study. Recovery of function was evaluated by the same battery of tests on days 77-79 of the study. At the end of the study (day 80), the animals were sacrificed for neurochemical assessments.

MAJOR FINDINGS AND PROPOSED COURSE: This experiment is still in progress.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The effects of repeated exposure to environmental contaminants such as kepone are of continuing concern to environmental health scientists. The systematic study of the toxicity of kepone is of concern because of the specific sequela of neurotoxicity observed in humans. Evaluation of substances such as kepone, which clearly affect human health, is clearly within the mandate of the Institute.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 90014-01 LBNT

PERIOD COVERED
August 22, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Effects of *In Utero* Exposure to PBBs on Neurobehavioral Development of Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT NIEHS
Other:	J. A. Moore	Associate Director	RRP NIEHS
	S. C. Bondy	Head, Neurochemistry Workgroup	LBNT NIEHS

COOPERATING UNITS (if any)
Research Resources Program, NIEHS

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.2	OTHER: 0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pregnant female mice were dosed every other day during gestation and until weaning with 3 or 10 mg/kg of PBBs. Male and female offspring were tested at 30, 90, and 120 days of age in a battery of neurobehavioral tests designed to assess a variety of sensorimotor functions and learning abilities. Binding characteristics of brain catecholamine systems were assessed.

PROJECT DESCRIPTION

METHODS EMPLOYED: Female C57Bl/6 mice were mated with male C_3H mice, two females per one male, for four or five consecutive days. Females were checked for sperm plugs daily. On the day plugs were observed, dosing with 0 (0.1 ml/10 g body weight of corn oil), 3, and 10 mg/kg of FF-1 began. The pregnant mice were dosed every other day during gestation and until weaning at 21 days of age. On day 4 after birth, all litters were standardized to five pups per litter.

At 30, 90, and 120 days of age the mice were assessed in a battery of tests including: body weights, startle to an air puff, motor coordination, forelimb grip strength, rectal temperature, motor activity, negative geotaxis, and emergence into a novel environment. Measure of one-way avoidance learning was also taken.

MAJOR FINDINGS AND PROPOSED COURSE: The experiment is still in progress. Depending upon the results of the neurobehavioral testing, binding characteristics of brain catecholamine system may be studied.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This work is related to the program of the Institute in that it provides basic research into an animal model of environmental disease. These studies are of particular importance since they are designed to assess the potential of an environmental pollutant such as the PBBs to affect the long-term behavioral development of animals exposed *in utero*.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90015-01 LBNT

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
The Effects of Repeated Administration of Acrylamide on Peripheral Sensation Using an Operant Titration Schedule

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. A. Burne	Psychologist	LBNT NIEHS
Other:	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.2	OTHER: 0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Acrylamide is used in the manufacturing of plastics and has been reported to produce alterations in peripheral sensory processes. The following work was designed to establish an animal model for the study of acrylamide-like alterations in somatosensory processes. Shock titration schedules have been used widely to assess peripheral sensitivity and analgesia in rats. Changes in reactivity to electric shock will be studied during and after repeated exposure to acrylamide.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: A plexiglas restraint tube equipped at one end with a nose poke detector and at the other end with a tail restrainer was developed. Shock was delivered to the rat's tail and incremented by 0.01 or 0.025 mA steps every 7.5 sec. Shock levels are reduced one step or reset to 0 by a nose poke response. Dosing with pharmacological standards to validate the procedure and with low doses of acrylamide is currently underway.

MAJOR FINDINGS AND PROPOSED COURSE: Pilot work has indicated that shock increment steps of 0.01 mA provided the most sensitive and stable baseline under the shock titration schedules. Following the establishment of stable baseline thresholds, rats will be dosed with acrylamide to look for altered sensitivity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This work is related to the program of the Institute in that it provides basic research into an animal model of environmental disease. These studies are of particular importance since they are designed to assess the potential of environmental pollutants on sensory processing in a rodent species. Such technology is clearly needed in behavioral toxicology.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 90016-01 LBNT

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Effects of Repeated Administration of Acrylamide on Motor Responses Under Control of Operant Schedules

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. A. Burne	Psychologist	LBNT NIEHS
Other:	H. A. Tilson	Head, Behavioral Toxicology Workgroup	LBNT NIEHS
	P. A. Cabe	Senior Staff Fellow	LBNT NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Behavioral Toxicology Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.2	OTHER: 0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Acrylamide is frequently used in the making of plastics and other consumer products. Overexposure to acrylamide has been reported to produce neuropathy due to distal axonal degeneration. In order to provide an animal model for acrylamide neurotoxicity, rats were trained on a lever press task at varied lever heights, followed by oral dosing with either 10 mg/kg acrylamide or distilled water five days per week for three weeks. Acrylamide impaired hindlimb neuromuscular functioning on raised levers. In addition, significant functional recovery following cessation of dosing was seen in acrylamide-treated rats.

POSITION DESCRIPTION

METHODS EMPLOYED: Male rats were dosed orally with acrylamide monomer (10 mg/kg) or with distilled water vehicle (1 ml/100 g). Doses were administered five days/week for three weeks. Rate of responding on a fixed-ratio 30 (FR30) schedule of reinforcement at two lever heights was recorded. Percent change in rate from predosing to dosing and from dosing to postdosing was analyzed for possible deficits on the high and low positioned lever. Responding on the lever in the high position was taken as a measure of hindlimb function. An additional measure of forelimb functioning using a force transducer is currently being implemented.

MAJOR FINDINGS AND PROPOSED COURSE: Dosing with acrylamide produced decreases in response rate at the high lever position but not at the low lever position. In addition, post reinforcement pause times (PRP), the amount of time it took for the rat to lower itself from the high lever to the food cup and return up, significantly increased in acrylamide-dosed rats. There was substantial recovery of function in the post dosing phase. No obvious signs of toxicity were observed. Through continued refinement of apparatus, the precise onset of neuromuscular toxicity due to repeated acrylamide dosing in the fore- and hindlimbs of rodents will be assessed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This work is related to the program of the Institute in that it provides basic research into an animal model of environmental disease. These studies are of particular importance since they are designed to assess the potential of an environmental pollutant such as acrylamide to affect the long-term behavioral performance of animals using sensitive operant behavioral techniques.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 90017-01 LBNT

PERIOD COVERED
March 1, 1979 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of Organic and Inorganic Derivatives of Heavy Metals on Binding of Spiroperidol to Striatal Membranes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. K. Agrawal	Visiting Fellow	LBNT NIEHS
Other:	S. C. Bondy	Head, Neurochemistry Workgroup	LBNT NIEHS
	S. B. Por	Visiting Fellow	LBNT NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Neurochemistry Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.0	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Many heavy metals have been shown to affect neurotransmitter function. Levels of neurotransmitters and their turnover rates have been found to be altered. No data exists on the effect of metals on postsynaptic binding events. This work involves measurement of effects of organic and inorganic derivatives of lead and mercury upon neurotransmitter receptors. This locus is a critical and often rate-limiting area of nerve function. In vivo and in vitro effects of these metals will be assayed. The developmental aspects of metal effects upon receptor characteristics is of special interest in view of the vulnerability of the immature nervous system to these neurotoxic compounds.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: As in previous project but with this addition: *In vitro* studies with heavy metal derivatives will be done over a large concentration range and the lowest levels of toxic agent to produce a detectable significant effect will be determined.

MAJOR FINDINGS AND PROPOSED COURSE: Preliminary data show a startling difference in the relative ability of polar and nonpolar lead compounds to inhibit the binding of radioactive spiroperidol to striatal membranes. The plans for future work fall into immediate and longer term goals. Our next experiments will be to study the effects of the toxic compounds we are using on the binding of a wider range of antagonist ligands for adrenergic and cholinergic receptors. After development of appropriate assays, we also plan to measure benzodiazpene, GABA, glycine, and glutamate binding sites.

Longer term goals will be the measurement of neurotransmitter translocations using brain membranes prepared from animals chronically treated with lead or mercury compounds. These will be developmental studies in that the progeny of exposed mothers will be used. Such ontogenic data will also be collected from young chicks exposed to heavy metals *in ovo*.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Heavy metal toxicity is a potential hazard to a large population in work or home situations. Both lead and mercury have been causative in causing permanent neurological damage after exposure. We have evidence that the dopamine neurons may exhibit a selective vulnerability to organic lead compounds, and that this may underlie some of the symptoms of lead poisoning. Our current data strongly imply that organic lead compounds may be unusually toxic and that this excess neurotoxicity cannot be solely related to increased penetrance through the blood brain barrier.

Other workers have attempted to account for lead toxicity in terms of competition with normal ions required for nerve conduction, such as calcium. It is essential to resolve this point since our data suggest that nonionic lead may be more hazardous than ionic lead and that ultimately, different acceptable levels may be needed for these two forms of heavy metal. This study is also a prototype that exemplifies two novel conceptual approaches: (a) the distinction between polar and nonpolar derivatives of a metal and (b) the emphasis on the synaptic movements of neurotransmitters rather than their overall levels.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 90018-01 LBNT

PERIOD COVERED
January 25, 1979 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Effect of Polyacrylamide on Striatal Nonamine Levels and Binding Site Density

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. K. Agrawal	Visiting Fellow	LBNT NIEHS
Other:	S. C. Bondy	Head, Neurochemistry Workgroup	LBNT NIEHS
	R. E. Squibb	Staff Fellow	LBNT NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Neurochemistry Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.8	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Acrylamide has long been known as a neurotoxin, deleterious to the peripheral nervous system. More recently this compound has been shown to have adverse effects on the morphology of the central nervous system. Since polyacrylamide poisoning is frequently associated with tremor, we are investigating the possibility that the locus of origin of some of these symptoms is the striatum. The levels of monamine neurotransmitters in the striatum of acrylamide-treated rats are being measured. The binding site characteristics of striatal membranes prepared from control and treated animals are measured using labeled ligands that are specific for a given receptor.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Membranes are prepared from the striatum by lysis and centrifugation. A combination of freezing, thawing, and osmotic shock is used to destroy morphological structure so that high affinity uptake mechanisms are rendered inoperative. These membranes are then incubated in the presence of very low concentrations (around 10^{-9}M) of labeled ligands that generally are antagonists of the neurotransmitter whose binding site is being studied. The proportion of ligand binding is determined after filtration and isolation of the membranes from the incubation medium. Nonspecific binding is estimated by repeating such an incubation in the presence of a relatively high concentration (10^{-4}M) of unlabeled ligand or other antagonist. Such methods enable the calculation of dissociation constants, binding site density, reversibility, association velocity, and cooperative interactions between binding sites. Levels of dopamine, norepinephrine, and serotonin are measured by flurometric means.

MAJOR FINDINGS AND PROPOSED COURSE: Initial studies have been directed toward the effect of relatively short-term (24 hr) exposure of rats to acrylamide. This condition significantly increases the apparent density of dopamine receptors in the striatal area by around 25%. Dose response studies suggest this effect becomes maximal at relatively low doses of acrylamide. The level of dopamine in striata of exposed animals is also increased. This is a paradoxical finding since excess receptor density is often consequent to denervation and depressed neurotransmitter levels.

The proposed course of this study is to extend our knowledge largely in two areas. Firstly, the effects of long term exposure to acrylamide will be contrasted to our short-term results. Chronic treatment may lead to either compensatory changes or an exacerbation of the lesion. Secondly, we will look objectively at a series of neurotransmitter binding sites in treated animals. This will establish whether the acrylamide is primarily toxic to a specific class of neurons. The next site to be measured will be the β-adrenergic receptor. Norepinephrine levels in the striatum of treated rats will also be assayed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM AT THE INSTITUTE: This work relates to the selectivity of acrylamide in its neurotoxic effects. It is relevant to regulation of acrylamide exposure, that its mode of action is better understood. The relation between the biochemical and the behavioral changes induced by acrylamide may be clarified by this study. It may be that the tremor associated with acrylamide poisoning, is related to abnormal dopamine metabolism, as is the case with Parkinson's Disease. This study may also provide a model for a new approach to the study of mechanisms of neurotoxicity of a variety of other compounds.

STANFORD RESEARCH INSTITUTE - Menlo Park, California 94025
(N01-ES-8-2154)

TITLE: Test Battery for the Neurobehavioral Assessment of Potential Neurotoxins

CONTRACTOR'S PROJECT DIRECTOR: Gordon T. Pryor, Ph.D.

PROJECT OFFICER (NIEHS): Hugh A. Tilson, Ph.D., Head, Behavioral Toxicology Workgroup, LBNT

DATE CONTRACT INITIATED: September 30, 1978

CURRENT ANNUAL LEVEL: $99,255

PROJECT DESCRIPTION

OBJECTIVES: The general objectives of the research are to (1) determine the steps necessary for selecting doses to be used in studies involving repeated administration of neurotoxic chemicals and comparison standards in low concentrations; (2) determine the ability of each of a set of neurobehavioral procedures, selected by NIEHS, to detect the presence of an expected neurotoxic effect and determine the capability of each test to provide a negative reading when an effect is not expected; and (3) generate a profile of effects for each substance so that tests assumed to measure the same or similar functions can be compared for relative sensitivity (eg., cumulative dose required to produce a significant effect).

METHODS EMPLOYED: Standardization and validation of neurobehavioral tests in rats shall be accomplished by evaluating the effects of the following chemicals: triethyl (or tetraethyl) tin, methyl mercury, sodium salicylate, d-amphetamine, acrylamide, polychlorinated biphenyl (Arochlor), arsenic, inorganic lead, and kepone.

The subjects are male albino rats of the Fisher-344 strain weighing approximately 200 g at the beginning of the study. The animals are housed individually in stainless steel cages in quarters having a constant light-dark cycle (light, 7 a.m. to 7 p.m.), temperature (21 ± 2°C), and relative humidity (50 ± 10%).

Each substance is being evaluated in three steps:

(1) The oral dose required to kill one-half of the subjects tested shall be determined (LD50). Mortality will be observed over a seven day period in at least five treatment groups.

(2) After the LD50 has been determined, a cumulative toxic dose (CTD) shall be ascertained in a 28 day, subacute toxicity study involving four groups of ten rats per group. Subjects in the three treatment groups will receive 1/2, 1/4, and 1/18 of the oral LD50 five days a week for four weeks. Those rats in the control group shall receive the vehicle. Daily measures of body weights and mortality shall be used to establish a CTD.

The CTD is defined as the dose producing $\leq 20\%$ mortality over 28 days of dosing.

(3) Once a CTD has been determined in the 28-day dose-ranging study, portions of the CTD (2/3, 1/3, and 1/6) will be given to another set of test animals by gavage five days per week for 90 days. The rats in each of the three treatment groups (n=10 per group) and those rats receiving vehicle (n=20 per group) shall be given a battery of tests the week prior to dosing (Predosing Phase), every third week during dose (Dosing Phase), and in the second and fourth weeks after cessation of dosing (Postdosing Phase). The total duration of the study shall be 120 days. The neurobehavioral assessments shall consist of two parts to be given on consecutive days during each week of testing. Tests include various measures of sensory and motor functioning and indicators of learning ability.

MAJOR FINDINGS AND PROPOSED COURSE: To date, the 7-day LD50 values for six of nine compounds and the CTD for three of nine compounds have been determined. A 90-day repeated dosing study for three compounds will soon be initiated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The Toxic Substances Control Act of 1976 requires that chemicals prevalent in the environment be evaluated for neurotoxicity. These include pharmaceutical, industrial, and agricultural chemicals and their by-products. Ideally, for such an evaluation a cost-effective battery of simple animal tests would be used, the reliability and validity of which had been demonstrated experimentally for known environmental toxins. However, a test battery that has general acceptance and that has been adequately validated is not currently available. The present research will help biomedical research in neurotoxicity by yielding a set of standardized tests and providing basic information concerning the neurotoxicity of the compounds being studied. Such information will be valuable for future studies on the mechanisms of action of these environmental agents.

LABORATORY OF BIOCHEMICAL GENETICS

LABORATORY OF BIOCHEMICAL GENETICS
Summary Statement

The primary objective of the program in the Laboratory of Biochemical Genetics is focused on development of systems to monitor the human population for induction of mutations in somatic and germinal cells and to evaluate the risk of exposure of the human population to environmental pollutants. The accomplishments of the goals of the Laboratory of Biochemical Genetics are vital for the general health of the human population. In order to reach the goals within the shortest possible time, several mechanisms are used. These include:

1. Development of an intramural research program based on in-house research by staff scientists and support of work at other institutions in the collaborative program with staff scientists as project officers.

2. Development of better mechanisms for information exchange. These include: (a) support of the Environmental Mutagen Information Center (EMIC) and (b) serving as associate editor or members of the editorial board of journals which publish research in the area of environmental mutagenesis.

3. Development of better mechanisms for training young scientists for research careers in environmental mutagenesis at: (a) the predoctoral level and (b) the postdoctoral level.

INTRAMURAL RESEARCH PROGRAM

The intramural staff has been organized into two programs. The major research accomplishments in each program are as follows:

In the Mutation Monitoring Group, the mechanism for induction of mutations in mammalian cells *in vivo* are studied. Several different selection and detection mechanisms are used for recognizing the mutant cells. Drs. Malling and Ansari are utilizing the various hemoglobins of the inbred strains of mice which differ in as many as thirteen amino acids. Strong monospecific antibodies have been produced to one of the types. The mutant cell can be detected by using the monospecific antibodies which are flagged with a fluorescent molecule. Sperm of mammalian species contain a specific lactic dehydrogenase-X (LDH-X). In collaboration with Mr. Burkhart this enzyme has been localized as an essential part of the tail. Interspecies antigenic differences are being utilized for detection of mutations. Drs. Ansari and Malling have developed the technique for detection of mouse sperm which react with monospecific antibody to rat LDH-X, indicating the presence of point mutations. This is the first time in history that anyone has detected a type of mutation in sperm which is likely to be point mutation. The frequency of this type of increase is almost proportional to the dose of procarbazine given intraperitoneally to mice. Dr. Malling is using histochemical methods to detect heat- and urea-resistant enzymes in individual sperm. Measurement of the frequency of abnormal sperm is used to screen environmental pollutants. Very little is known, however, about the mechanism which results in an abnormal sperm. To at least get a handle on this problem, Mr. Burkhart and Dr. Malling have studied the genetics and the ultrastructural mechanism of one particular sperm mutation.

The work of the Physiological Genetics Group is concerned with the development of methods for detecting germinal mutations and with the consequences of germinal mutations. Dr. Johnson is presently screening offspring of animals treated with procarbazine. Electrophoresis is one technique presently being investigated for its ability to resolve mutations in mice. Three mutants, partially confirmed by genetic analysis, have been identified so far in present experiments. These mutations result in altered enzymes -- malic enzyme, isocitrate dehydrogenase, and phosphoglucomutatase.

In the biochemical specific locus mutation system, offspring of animals treated with procarbazine are presently being screened for presence of mutations. Dr. Johnson is developing a long series of additional tests on the micro centrifugal fast analyzer which can be done simultaneously with the electrophoretic analysis.

COLLABORATIVE RESEARCH PROGRAM

In the Collaborative Research Program most of the contracts fall naturally within two areas: (1) development of systems for monitoring of the human population for mutations and (2) dissemination of mutagenesis information. The contracts will be described following this outline.

1. Mutation monitoring systems for the human population - In a contract at the University of Washington, an attempt is being made to develop a simple system to measure point mutations in readily accessible human somatic cells. This assay is based on changes in the expected frequency of human red blood cells containing mutant hemoglobin rather than normal hemoglobin. Specific antisera are being developed to detect red blood cells, each of which carry one of ten different hemoglobins. By conjugating the antiserum with different colored fluorescent dye, mutant RBC's will fluoresce.

2. Collection and dissemination of mutagenesis literature - During the past year we have continued to support the Environmental Mutagen Information Center (EMIC) by interagency agreement at the Oak Ridge National Laboratory. This center has over 24,000 bibliographic entries in its data banks and contains as a unique world-wide resource for information in the area of environmental chemical mutagenesis.

INFORMATION EXCHANGE

Journals - Staff members serve as associate editors, assistant editors, or members of editorial boards of Mutation Research, Environmental Health Perspectives, The Journal of Toxicology, and Reviews in Genetic Toxicology. This makes it possible not only to promote publication of papers dealing with various research areas of environmental mutagenesis but also to promote development of new methods for publication.

TRAINING PROGRAMS

The great shortage of scientists to do research in the area of environmental mutagenesis both in the United States and abroad has provided incentive for staff scientists to develop a training program at both the predoctoral and postdoctoral levels. At the postdoctoral level, staff scientists included three visiting fellows from two foreign countries.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 65002-02 LBG

PERIOD COVERED
October 1, 1978, through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Mutation Studies using Hemoglobin and Monospecific Antibodies Against Hemoglobins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. A. Ansari	Visiting Scientist	LBG	NIEHS
Other:	H. V. Malling	Laboratory Chief	LBG	NIEHS
	M. A. Baig	Visiting Fellow	LBG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Mutation Monitoring Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is to develop and test systems that could be used to study mutagenesis in mammals using single cells. Monospecific antibody against mouse hemoglobins has been developed. Techniques have been worked out for making red cell smears and fixing the cells quantitatively for staining.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Monospecific antibody against different mouse hemoglobin variants is prepared. The purified antibody has the characteristic of interacting with the hemoglobin from one strain only (e.g., C57BL/6 and not from other strains such as DBA/2). Such an antibody preparation is used to label red blood cells from DBA/2 mice by a sandwich fluorescent antibody technique. Normal DBA/2 cells do not show any fluorescent label whereas mutant cells are brightly stained.

MAJOR FINDINGS AND PROPOSED COURSE:

1. Preparation of monospecific antibody. In the initial studies, ten horses were immunized with hemoglobin s (C57BL/6) and hemoglobin d_{maj} (DBA/2). The antisera were found to contain high titers of antibodies against the hemoglobins. However, as expected, most of these antibodies were directed against determinants common between s and d_{maj} hemoglobins and therefore were cross-reacting. Attempts to purify monospecific antibodies from these sera by absorption on hemoglobin immunoabsorbents resulted in poor yield. Moreover, such purified antibodies were not strong enough to give adequate immunofluorescence. Fifty antisera obtained from different bleedings of these immunized horses were screened for the presence of monospecific antibody by the fluoresent bead technique developed in this laboratory (Project No. Z01-ES-65005-01 LBG). None of these antisera was found to contain monospecific antibody good enough for the immunofluorescence work. Next, twenty rabbits were immunized with s and d_{maj} hemoglobins. All the rabbits developed high titer antibodies. However, when screened by the fluorescent bead technique, none was found to give monospecific antibody. In the third attempt immunization of mice with mouse hemoglobins was undertaken. Hemoglobins from C57BL/6 (hemoglobin s), DBA/2 (d_{maj}) and SJL (hemoglobin s) were injected into 21 groups of mice of various strains differing at the hemoglobin gene loci. The recipient strains included: DBA/2, AU/Ss, CXBG/By, CXBI/By, SEC/1Re, C58, C3HeB/Fe, PL, LP, C57BL/6, C57BR/Cd, SJL, Balb/c, SM, and SWR. Out of all these groups, LP mice immunized with C57BL/6 hemoglobin gave an antibody that reacts only with C57BL/6 red cells and not with DBA/2 cells. This monospecific antibody gives strong immunofluorescence and can be used to pick up mutation from d_{maj} to s hemoglobin in DBA/2 mice.

2. Smear preparation. The objective is to put a known number of cells on the slide, fix them quantitatively, stain with the fluorescent antibody and then scan the slide to locate the few fluorescent (mutant) cells. If there has not been a significant loss of the red cells, the total number of cells on the slide would already be known, and only the mutant cells will have to be counted to calculate the mutation frequency. Proper medium and dilution of the red cells seem to be an important factor for making satisfactory smears for this purpose. Washed cells in plain phosphate buffered saline (PBS) make very poor smears; the cells lose their natural round shape. Addition of fetal calf serum (FCS) to the suspension takes care of this problem. A mixture of washed cells, FCS, and PBS in the ratio of 1:1:4 has been found to be most satisfactory giving monolayer, even distribution; higher concentrations of

the cell suspension result in uneven cell distribution and some times multilayered smears, whereas too dilute a mixture results in smears with too little cells.

3. Fixation. The next step is to fix the cells such that all the cells would adhere to the slide and would give proper staining with the antibody. The smears are blow dried at room temperature for 1 hr, treated with fixing solvent, washed with PBS and distilled water and finally staining is started. Several fixing solvents were tried. Some of them cause loss of majority of the cells while others make the cell membrane rigid and impermeable to the antibody. Some of the interesting observations in this connection are noted here. Acetone-ethanol mixture (85:15) is excellent for subsequent staining but causes loss of cells. Precoating the slides with 1% agar, or 1% gelatin or 1% solution of Parlodion in ethanol-ether mixture (1:1) does not prevent the loss of the cells. Treatment of the dried smear with formalin vapor (10 sec. to 1 min) before fixation in the Acetone-ethanol mixture did help the cells to stick on the slide but at the same time made the cell wall too rigid to open up for the antibody, resulting in loss of staining. Acetone alone does not fix the cells at all (all the cells are washed off). Ethanol and methanol alone are good fixatives by themselves but do not open up the membrane. Acetone-methanol mixture (15:15) is an excellent fixative but staining is much weaker as compared to the acetone-ethanol mixture. When the smear was first treated with 100% or 70% methanol for 1 or 5 min followed by treatment with acetone-ethanol mixture (85:15) cells were found to be fixed but staining was even poorer. It appears from these studies that (1) acetone is required to open the membrane permeable to the antibody, (2) methanol or ethanol is needed to fix the cells, (3) methanol is a better fixative than ethanol but the latter is better for subsequent staining. A mixture of acetone-ethanol-methanol (80:10:10) was found to be best suited for our purposes. Time of fixation has profound effect on the brightness of staining. In general increasing time of fixation from 5 min to 45 min causes an increase in the fluorescent staining. Further increase in time is too much deleterious upon the cell membrane and may cause complete disappearance of the membrane.

4. Proposed course. Using this monospecific antibody mutation frequency, spontaneous as well as after treatment with several known and unknown mutagens, will be determined. This system could also be developed into a screening system for mutagenic activity of chemials. Monospecific antibodies will also be developed that react with only monkey hemoglobin and not with human hemoglobin. Such antibodies will be used to study mutation from human to monkey type hemoglobin, and the system could be used as a monitoring system for human population.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND PROGRAM OF THE INSTITUTE: One of the major goals of the Laboratory of Biochemical Genetics is to develop and test systems that could be used to screen chemicals and pollutants for their mutagenic and/or carcinogenic action directly in mammals using single cells. Another goal is to develop methods suitable for monitoring human population for any genetic alterations in exposed individuals. This project is aimed at developing a method that would serve both these goals screening as well as monitoring.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 65007-02 LBG

PERIOD COVERED
October 1, 1978, through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Study of mutation by using sperm specific enzyme LDH-X

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. A. Ansari	Visiting Scientist	LBG	NIEHS
Other:	H. V. Malling	Chief, LBG	LBG	NIEHS
	L. M. Bahuguna	Visiting Fellow	LBG	NIEHS
	J. G. Burkhart	Research Chemist	LBG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Mutation Monitoring Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was undertaken to study mutagenesis using monospecific antibodies against the sperm specific enzyme, lactate dehydrogenase-X. A monospecific antibody has been prepared from rabbit anti-serum that reacts with rat sperm and not with mouse sperm. Using this antibody in a sandwich fluorescent antibody technique, the frequency of spontaneous mutation of mouse LDH-X in the DBA/2 mouse to "rat kind" LDH-X molecule has been estimated to be 0.4×10^{-6}. The induced mutation frequency shows an upward trend upon treatment of the mice with increasing dosage of procarbazine.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED:

1. Preparation of monospecific antibody. The gamma globulin fraction from a rabbit antiserum against rat LDH-X is isolated by precipitation with 18% sodium sulfate. After dialysis against phosphate-buffered saline (PBS), the antibody is absorbed with the following materials in sequence to remove the cross-reacting antibody molecules: plain Sepharose-4B, mouse LDH-X immunoabsorbent, and mouse sperm. The absorbed preparation is checked for its monospecificity as indicated by its reaction with rat sperm and no reaction with mouse sperm.

2. Labeling technique. Fresh mouse sperm from vasa deferens are washed two times with PBS and suspended in 1 ml of the same buffer. Twenty microliters of the monospecific antibody is added and the mixture incubated for three hours at 4°C. The unreacted antibody is washed off three times with cold PBS and the sperm are again suspended in 1 ml of the buffer. Fifty microliters of a second antibody (1 mg/ml), fluorescein isothiocyanate-coupled goat anti-rabbit IgG, is then added and allowed to react for 3 hrs at 4°C. After washing and suspending, sperm concentration in the suspension is determined by the use of a hemacytometer. Fifty microliter aliquots of this suspension, containing 1 to 5 million sperm, are spread in the center of microscopic slides in the form of a rectangle approximately 2.5 cm x 1.3 cm in size.

3. Locating and counting the mutant sperm. The entire area of the slide containing 1 to 5 million sperm is scanned under a fluorescent microscope equipped with epi-illumination system. Sperm that are brightly fluorescent are counted as mutants.

MAJOR FINDINGS AND PROPOSED COURSE:

1. Localization of the enzyme LDH-X on the sperm surface. During the course of this study we found that if the unpurified anti LDH-X serum is used, the entire sperm, including head, midpiece, and tail, is stained. This kind of staining is due to the presence of some natural antibodies in the serum against carbohydrates that interact with the sperm membrane. After removing these natural antibodies and purification as described under the methods section, the purified antibody gives fluorescence only in the tail portion, the head and the midpiece are not stained. Thus, we have unequivocally demonstrated that so far as the outer surface of the sperm is concerned, the enzyme is present only in the tail and not on the head and the midpiece. This observation makes biological sense, because the enzyme is present where it is needed most.

2. Demonstration that the fluorescent staining is due to the intrinsic enzyme of a sperm and not due to enzyme just picked up by the sperm from semen. The fact that only tail is stained itself indicates that the staining is not due to enzyme picked up by the sperm from the semen. Moreover, the sperm is

washed before the staining procedure starts. To further prove this point, rat and mouse sperm were mixed in different proportions ranging from 1:1 to 1:100,000 and then stained with the monospecific antibody that reacts only with rat LDH-X and not with mouse LDH-X. In the mixture only rat sperm were found to stain, no mouse sperm were stained. If the sperm were picking up some enzyme from the medium some mouse sperm would also have been labeled.

3. Mutation frequency. The monospecific antibody used in these studies interacts with rat LDH-X and not with mouse LDH-X. However, if the mouse LDH-X undergoes mutation to generate rat kind LDH-X this antibody would interact with the mutant molecule. Thus, normal mouse sperm would not be stained but the mutant mouse sperm will be fluorescent.

We found 72 such mutant sperm out of 167×10^6 sperm from normal DBA/2 mice giving a spontaneous mutation frequency of 0.43×10^{-6}. Mice treated with increasing dosage of procarbazine showed an upward trend in the mutation frequency; the procarbazine dose (mg/kg) and corresponding mutation frequency are as follows: 200, 1.94×10^{-6}; 400, 3.66×10^{-6}; 600, 5.47×10^{-6} and 800, 9.17×10^{-6}. These data represent an almost linear dose vs. response relationship.

4. Proposed course: (1) Automation. Scanning the slides for locating the mutant sperm is a very tiring and time-consuming task. A computerized automatic scanning system will be developed. (2) More mutagens. Dose vs. response relationship has been obtained for procarbazine. Similar experiments will be done using other known mutagens, e.g., mitomycin C and TEM. After it has been demonstrated that the system works with known mutagens, it will be tried with chemicals that are suspected mutagens or carcinogens. (3) Screening systems. It is hoped that after several known and unknown mutagens have been successfully tested with the sperm system, the method could be accepted as a screening system for mutagenic activity of chemicals. This will be an *in vivo* mammalian screening system. (4) Monitoring system. Monospecific antibodies will be developed that would react only with monkey LDH-X and not with human LDH-X. Such antibodies will be used to study mutation from human to monkey type LDH-X. The utility of this system would be for monitoring human population for genetic changes.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND PROGRAM OF THE INSTITUTE: These studies are part of the Institute's program to develop and test systems that could be used to study mutation in mammals *in vivo* using single cells. When fully developed this system could be used to screen chemicals for their mutagenic/carcinogenic activity and to monitor human population for any genetic alterations.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65008-01 LBG

PERIOD COVERED
October 1, 1978, through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Mechanisms of Sperm Morphology Mutations in PL Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. G. Burkhart	Research Chemist	LBG	NIEHS
Other:	H. V. Malling	Laboratory Chief	LBG	NIEHS

COOPERATING UNITS (if any)
Environmental Biology and Chemistry Branch

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Mutation Monitoring Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.1	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project was to provide understanding about the mechanisms of sperm differentiation that may be related to induction of sperm morphology mutations in mice and humans. Homozygous PL mice produce a wide range of normal and abnormal sperm. We are studying (1) the comparative morphology of normal and abnormal sperm, (2) ultrastructural mechanisms of differentiation that cause the morphology mutations, and (3) the inheritance of the trait.

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: A high frequency of sperm from the inbred homozygous PL mice range are abnormal in comparison with other inbred strains. Some of the abnormalities involve misshapen heads; however, a 27.5% of the sperm produced are headless and have mitochondria that are condensed into a mass at the caudal end of the midpiece region; all sperm with tails exhibit relatively normal motility. The frequency of the headless variants in heterozygous F_1 and F_2 progeny indicates the characteristic is mainly recessive but influenced by the female. Electron microscopy of the testes provides evidence that the abnormal sperm morphologies in PL males results from a failure of the paired centrioles to attain a normal position on the nucleus relative to the acrosome before implantation. Future investigations in this area will involve correlation of sperm morphology mutations with quantitative DNA measurements and treatment with mutagenic agents.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAMS OF THE INSTITUTE: Sperm abnormality can be induced by treatment with mutagenic agents. Measurement of the frequency of abnormal sperm in mice and humans has potential as an indicator of exposure to harmful environmental agents. This study is important because it will provide useful information about the ultrastructural mechanisms of sperm differentiation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 65009-01 LBG

PERIOD COVERED
October 1, 1978, through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Detection of Translocation Carriers by DNA Measurement in Sperm

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. G. Burkhart	Research Chemist	LBG	NIEHS
Other:	H. V. Malling	Laboratory Chief	LBG	NIEHS
	T. G. Riddle	Biomedical Engineer	Contract Support	

COOPERATING UNITS (if any)
Environmental Biology and Chemistry Branch
Biometry Branch

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Mutation Monitoring Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS: 1.2	PROFESSIONAL: 0.2	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to develop methods to detect translocation carriers in populations of mice treated with potential mutagens by evaluating the DNA distributions of sperm. The Axiomat scanning microscope is being used to quantitate the DNA fluorescence of single sperm and spermatids. The measured distributions of DNA fluorescence are being evaluated within and between normal mice and those carrying known translocations. Work has begun to examine the feasability of automatic systems of measurement and analysis.

PROJECT DESCRIPTION

METHODS EMPLOYED: Meiosis in male translocation carriers results in sperm that have normal and abnormal amounts of DNA. Fluorescent labels can be used to quantitate DNA by measuring the relative intensity of emitted light upon excitation of the fluorochrome. In principle, the variance of the distribution describing DNA fluorescence of sperm populations will be greater in individuals with translocations. Fluorescence will be measured with the Axiomat microscope. Data on single cells and populations will be collected and analyzed by the PDP-11 computer.

MAJOR FINDINGS AND PROPOSED COURSE: At present it has been possible to measure DNA distributions of mature sperm in normal DBA/2J mice and in special strains that carry Robertsonian translocations. The variances of the DNA fluorescence distributions in sperm populations have been found to be significantly greater in the translocation carriers in comparison with normal DBA/2J mice. Future studies will be directed towards maximizing the resolution of the system. In addition F_1 males derived from parents treated with compounds known to induce chromosome abnormalities will be evaluated. Data will be correlated to other tests used to detect translocations such as the fertility test and cytogenetic analysis.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Development of this system would significantly reduce the time cost and animals needed to assess the potential of compounds to induce chromosomal damage.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 65010-01 LBG

PERIOD COVERED
October 1, 1978, through September 30, 1979

TITLE OF PROJECT (80 characters or less)
Translocation and sperm abnormality induction by N-Isopropyl-α-(2-methylhydrazino)-p-toluamide hydrochloride (procarbazine hydrochloride) in mouse spermatogonia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. K. Sharma	Visiting Fellow	LBG	NIEHS
	G. T. Roberts	Visiting Fellow	LBG	NIEHS
	F. M. Johnson	Research Geneticist	LBG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Physiological Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The present study was attempted to test whether the treatment of spermatogonial cells with procarbazine induces reciprocal translocations or sperm abnormalities. No translocations were observed in 840 spermatocytes of control animals or 680 cells of animals treated with 400 mg/kg. Only one translocation was observed among 1000 spermatocytes of animals treated with 100 mg/kg. A significant increase in the proportion of sperm with abnormal heads was observed at 400 mg/kg but not at 100 mg/kg.

PROJECT DESCRIPTION

METHODS EMPLOYED AND MAJOR FINDINGS: Ten to twelve week-old DBA/2J males were injected intraperitoneally with either procarbazine dissolved in sterile normal saline (treated animals; doses 100 mg/kg and 400 mg/kg) or with sterile saline only (control animals). For the translocation tests, animals were sacrificed by cervical dislocation after 57-63 days of injection. Meiotic preparations from testes were made by an air drying technique and dividing spermatocytes were examined for multivalent configurations. For the sperm abnormality tests, sperm from vas deferens were stained with eosin Y and examined for abnormality in head morphology 52 days after injection. No translocations were observed in 840 spermatocytes of control animals or 680 cells of animals treated with 400 mg/kg. Only one translocation was observed among 1000 spermatocytes of animals treated with 100 mg/kg. A significant increase in the proportion of sperm with abnormal heads was observed at 400 mg/kg but not at 100 mg/kg.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Genetic damage induced at spermatogonial cell stage is of great significance because spermatogonia represent the permanent cell population in the testis. Reciprocal translocations constitute an important category of genetic damage because they can be transmitted from generation to generation besides leading to reduction in fertility. Many diseases are associated with translocations. In recent years, elevated sperm abnormalities have been found when mutagenic substances were tested; hence, the sperm abnormality assay has been suggested for assessing genetic damage in individuals exposed to chemicals and pollutants. This study indicates that at a higher dose, procarbazine causes sperm abnormalities although there is no proof that it can induce reciprocal translocations.

PUBLICATIONS

Sharma, R. K., Roberts, G. T., Johnson, F. M., and Malling, H. V.: Translocation and sperm abnormality assays in mouse spermatogonia treated with procarbazine. Mutation Res., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60019-06 LBG

PERIOD COVERED
October 1, 1978, through September 30, 1979

TITLE OF PROJECT (80 characters or less)
The Use of Male Germinal Tissue in the Detection of Mutational Events

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. V. Malling	Laboratory Chief	LBG	NIEHS
Other:	M. Snell	Bio. Lab. Tech.	LBG	NIEHS

COOPERATING UNITS (if any)
Environmental Biology & Chemistry Branch
Biometry Branch

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Mutation Monitoring Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.2	0.8

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
The aim of this project is to develop systems for measuring the frequency of point mutations in male germinal tissue. These systems would have the advantage of testing the human population by means of readily available samples, i.e., spermatozoa. At present enzyme histochemical methods are being developed to detect point mutations in male mice. The histochemical staining technique has been quantatized for α-glycerophosphate dehydrogenase and succinyl dehydrogenase. Techniques have been developed for selective staining of sperm containing thermoresistant or urea-resistant α-glycerophosphate dehydrogenase. Mutation frequencies have been determined after treatment of the male mice with procarbazine.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Some enzymes in sperm can be detected readily by histochemical methods. The enzymes under study in this laboratory are α-glycerophosphate dehydrogenase and succinate dehydrogenase.

The principle of these studies is that, with the use of standardized staining procedures and use of urea thermodenaturation, it should be feasible to detect thermo-resistant mutations in the spermatozoal enzymes. These mutations will be estimated by counting stained and unstained cells with the aid of a Zeiss Axiomat microscope connected to a PDP-12 computer and a teleprinter output. Information also will be collected on magnetic tape for processing in the Institute's PDP-11 computer.

MAJOR FINDINGS AND PROPOSED COURSE: Thus far we have developed histochemical methods for α-glycerophosphate dehydrogenase (α-GPD) and succinic dehydrogenase. The dehydrogenases were localized by using nitro blue tetrazolium. The accumulation of stain in the single midpiece was measured directly by use of a Zeiss Axiomat microscope and a photomultiplier tube. The accumulation of stain was proportional to time of staining up to 40 minutes. Variation of substrate concentration, staining temperature, and pH indicated that the enzyme had expected enzyme properties *in situ*. In sperm from DBA/2J mice the half-life of α-GPD at 60°C was 42 minutes and at 63°C was 18 minutes. *In vitro* the half-life at 63°C was 8 minutes, indicating that there was only a slight difference in the thermolability for α-GPD *in situ* and *in vitro*. The α-GPD in sperm from C57BL/6J mice was slightly more sensitive to the heat treatment *in situ* than the same enzyme in DBA/2J mice. After heating of sperm from DBA/2J mice to 63°C for 60 minutes most sperm do not stain at all; however, a few sperm were stained strongly. It is possible that these sperm could be mutants containing thermo-resistant enzymes. Similar techniques have been developed for detection of sperm containing α-glycerophosphate dehydrogenase resistant to urea denaturation. The frequency sperm with urea-resistant α-GPD increased with increasing doses of procarbazine. The development of the techniques for measuring the mutation frequencies has just started, and work in this area will be continued. Mutation frequencies for thermo-resistance and urea-resistance will be determined after treatment of mice with a series of standard mutagens.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Development of this system is important, because it would permit the detection of mutational events within a single individual that could be used to monitor the human population for mutations in the male germinal cells.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60021-07 LBG

PERIOD COVERED
October 1, 1978, through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Gene Mutation Indicators in Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. M. Johnson	Research Geneticist	LBG	NIEHS
Other:	R. K. Sharma	Visiting Fellow	LBG	NIEHS

COOPERATING UNITS (if any)
Research Triangle Institute, Divisions of Biology and Analytical Sciences, Research Triangle Park, NC; North Carolina State University, Depts. of Statistics and Animal Science, Raleigh, NC

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Physiological Genetics Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Electrophoretic methods, spectrophotometrically determined enzyme characteristics, morphometric features, and metabolite profiles are being investigated for their potential to indicate newly occurring mutational events in mice.

PROJECT DESCRIPTION

OBJECTIVES: The objective of this work is the development of methods to detect germinal mutations in mice. Characteristics and methods which are being explored are enzyme properties as reflected by starch gel electrophoresis and isoelectric focusing, enzyme activities determined by centrifugal fast analyzer, metabolic profiles analyzed by gas chromatography and mass spectrometer, and physical dimensions of the skeleton measured with the aid of an X-Y digitizer/computer system. Male mice, some of which are mutagen treated, are mated with females and F_1 offspring obtained. Tissue samples are removed surgically from the parental and F_1 animals and subjected to analysis. Suspected mutants are mated to confirm the genetic basis of variants. Mutants affecting phosphoglucomutase, malic enzyme, isocitrate dehydrogenases and hemoglobin have been found. After confirmation, mutant gene products are characterized biochemically. Later, the impact of the mutations on the life functions of the animals will be investigated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The significance to human health of germinal mutations and increased germinal mutation rates is not well understood. Two needs are critical if the question is to be probed effectively, (1) a large, efficient and economical series of methods capable of detecting various classes of mutational events in material sampled directly from humans and (2) a whole animal model system which can be manipulated experimentally. The mouse, the animal with which this project is directly concerned, provides a convenient source of material for methods development; and because of reasonable homology with man, at least some of the methods used for mice, plus some of the mutations and effects observed for mice, can be expected to be relatable to humans. As methods are provided with the mouse, model system capability is also generated. With this it should be possible to test directly the genetic effects of specific environmental mutagens and explore generally the fundamental relationships concerning mutations and their impact on fitness and health.

THE JACKSON LABORATORY - BAR HARBOR, MAINE
(NIH-N01-ES-5-2159)

TITLE: Development of a New Mouse Strain to Maximize the Sensitivity of a Point Mutation Assay

CONTRACTOR'S PROJECT DIRECTOR: Thomas H. Roderick, Ph.D.

PROJECT OFFICER (NIEHS): F. M. Johnson, Ph.D.

DATE CONTRACT INITIATED: February 19, 1974

CURRENT ANNUAL LEVEL: $42,000

PROJECT DESCRIPTION

This contract is for the construction of a special strain of mice which differs to the maximum practical extent for alleles at many loci from the standard DBA/2J strain. Variant alleles not found in either DBA/2J or C57BL/6J are introduced into the C57BL/6J background genotype by mating and repeated backcrossing, the latter being conducted with appropriate monitoring to assure the continued presence of existing allelic differences between the two strains. Electrophoretically expressed enzyme characteristics are presently being utilized. A series of matings and backcrosses have been conducted, but a strain with any significant advantage over C57BL/6J is yet to be developed.

There is no practical system presently available with which it is possible to detect systematically and efficiently newly induced point mutations and to study their biological effects in mammals. This contract serves the purpose of assisting the development of such a capability.

INFORMATION CENTER COMPLEX/INFORMATION DIVISION
OAK RIDGE NATIONAL LABORATORY - OAK RIDGE, TENNESSEE 37830
(Interagency Agreement Between NIEHS and ORNL)

TITLE: Environmental Mutagen Information Center

CONTRACTOR'S PROJECT DIRECTOR: Mr. John S. Wassom

PROJECT OFFICER (NIEHS): H. V. Malling, Ph.D.

COLLABORATING INSTITUTES: National Cancer Institute; Energy Research and Development Administration

DATE CONTRACT INITIATED: July 1, 1970

CURRENT ANNUAL LEVEL: $210,000

PROJECT DESCRIPTION

OBJECTIVES: The mission of the Environmental Mutagen Information Center (EMIC) is to collect, organize, and disseminate chemical mutagenesis information.

MAJOR FINDINGS AND PROPOSED COURSE: EMIC's data file at the end of March 1979 contained 23,000 bibliographic entries. Of these, approximately 22,500 have been indexed with respect to agent(s), organism(s), and Chemical Abstract Service Registry Number(s). The Center processes requests for information at a rate of 2-3 per day. These requests come primarily from governmental agencies in the United States or government supported research in universities. Several requests from other countries are also received. The EMIC data bank can now be screened by Tox-Line. EMIC will continue to monitor the world scientific literature for reports on chemical mutagenesis. It will issue its annual literature survey in the middle of the fiscal year. This report will include an index to test organisms or test objectives as well as indexes for agents and selected title keywords. As great a portion as possible of EMIC's total bibliographic entries will be keyworded with respect to agent, test organism, and test object. EMIC will continue to answer selected requests for special information for the scientific, educational, and industrial communities. Tabular extracts of the data from literature will be added to the data file as they are prepared in conjunction with the timely state-of-the-art reports on subjects of current scientific interests.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many compounds with widespread distribution in man's environment are known to be mutagenic in laboratory test organisms. Man's constant contact with mutagens may pose more danger to the genetic health of the human population than does radiation. The awareness of this problem among scientists, administrators, and laymen created a need to make reference to published reports on tests for chemical mutagenesis readily available. This is important because these reports are scattered throughout a great number of publication sources (1550). EMIC has helped several national commissions in collecting and extracting the data from the literature. For the Institute, and especially the Laboratory of Biochemical Genetics, EMIC saves more than one man-year of time for the personnel in the Laboratory by supplying their information needs.

PUBLICATIONS

Wassom, J. S. and Malling, H. V. "Specialized Information Centers in Toxicology," Chapter 11 in Advances in Modern Toxicology, Hemisphere Publishing Corp., Washington, D.C., pp. 349-383 (1978).

Wassom, J. S., Huff, J. E., and Loprieno, N. "The Genetic Toxicology of Chlorinated Dibenzo-p-dioxins," Mutation Res. (in press).

Wassom, J. S., Shelby, M. D., Von Halle, E. S., Beauchamp, R. O., Jr., Owens, E. T., Miller, I. R., Barnard, W. J., and Adams, J. O. Chemical Mutagenesis: A Survey of the 1975/1976 Literature. Oak Ridge National Laboratory Publication No. ORNL/EMIC-10. Oak Ridge National Laboratory, Oak Ridge, TN, pp. 502 (1978).

Wassom, J. S., Shelby, M. D., and Von Halle, E. S. Mutagenesis and Teratogenesis Information Programs. In, Proceedings of the Symposium on the Handling of Toxicology Information, Bethesda, MD (in press).

Wassom, J. S. and Malling, H. V. Specialized Information in Toxicology: I. Environmental Mutagen Information Center. In, Advances in Modern Toxicology (Eds.) W. G. Flamm and M. A. Mehlman (1978).

Brown, M. L., Wassom, J. S., Malling, H. V., Shelby, M. D., and Von Halle, E. S. Non-mammalian assay systems used in the evaluation of selected chemical compounds for mutagenic activity/A literature summary. J. Natl. Cancer Inst. 62: 841-871, 1979.

GEORGIA INSTITUTE OF TECHNOLOGY - ATLANTA, GA 30332
(NIH-N01-ES-5-2135)

TITLE: Development of a Polygenic Assay for Point Mutations in Mice

CONTRACTOR'S PROJECT DIRECTOR: John W. Crenshaw, Jr., Ph.D.

PROJECT OFFICER (NIEHS): F. M. Johnson, Ph.D., Research Geneticist

DATE CONTRACT INITIATED: June 26, 1975

CURRENT ANNUAL LEVEL: $76,500

PROJECT DESCRIPTION

The purpose of this contract is to investigate mutagenic effects in mice of some known mutagens, as measured by mean and variance changes in polygenic characters. The contractor is investigating the effects of mutagen-induced polygenic mutations on a series of traits in F_1 and F_2 generation progenies descended from mutagenized males of inbred strains of mice. Progeny are examined for traits such as age of eruption of incisors, body weight, sex ratio, righting response, and hematocrit. Different strains and mutagens are studied. Results have been mixed in experiments conducted to date. Characteristics subject to change as a result of mutation at any of a number loci may be useful to the detection of mutagenic activity associated with environmental substances. This approach is in contrast with other work which attempts to detect mutagenic change in characters controlled by individual specific genes.

UNIVERSITY OF WASHINGTON - SEATTLE, WASHINGTON
(NIH-NIEHS-74-2151)

TITLE: Detection of Point Mutations in Somatic Cells

CONTRACTOR'S PROJECT DIRECTOR: G. Stamatoyannopoulos, M.D.

PROJECT OFFICER (NIEHS): Heinrich V. Malling, Ph.D.

DATE CONTRACT INITIATED: January 1, 1974

CURRENT ANNUAL LEVEL: $192,000

PROJECT DESCRIPTION

OBJECTIVES: It is likely that an increase in the mutation rate in the human population would be detrimental, yet there is no system developed to monitor the human population for point mutations. The purpose of this investigation is to develop a simple system to measure point mutations in readily accessible human somatic cells. It is likely that the somatic cell mutation rate would reflect the germ cell mutation rate, and the rate of introduction of genetic defects into the human population.

METHODS EMPLOYED: Many different types of hemoglobin mutations exist in the human population. Some of these are point mutations in the α- and β-chain. Since such mutations occur in the germinal tissue, it is reasonable to assume that they also occur in the stem cells for the red blood cells (RBC). This type of mutation should result in an RBC which would contain an aberrant hemoglobin. By using a monospecific antibody to a number of aberrant hemoglobin types, such cells should be detected in samples from normal individuals after reaction of the antibodies with various fluorescent dyes. Antibodies are being produced against HbS, HbC, HbOArab, HbE, HbQ India, Hb Ottawa, Hb Hasharon, Hb Cranston, Hb Wayne, and Hb Constant Spring.

MAJOR FINDINGS AND PROPOSED COURSE: Most of the monospecific antibodies have been produced against variants of hemoglobins due to base-pair substitutions. Hemoglobin Wayne is an α-chain frameshift mutant. Production of antibodies against this hemoglobin variant have been difficult. Finally, antibodies of satisfactory quality were obtained. There is no reaction of the fluorescent antibody with the Hb A containing cells, there is fluorescent labeling of the Wayne cells and a dimorphic appearance of the artificial AA/Wayne mixtures. However, in contrast to the anti-S or anti-C antibodies in which there is small variation between cells in fluorescent intensity, a considerable degree of variation in fluorescent intensity was noted in the preparation containing Hb Wayne cells. There are two possibilities for this variation which will be investigated; namely, (a) the variation is due to cell age related decrease in the Hb Wayne content or (b) the variation in the Hb Wayne red cell content expresses reciprocal relationship between HbF and Hb Wayne synthesis per red cell.

An effort has been made to determine the number of red cells binding the anti-Hb-S-FITC. The average frequency of Hb-S containing cells in normal individuals are 1.1 x 10^{-7}. Attempts have been made to determine this frequency in abnormal situations. Cell screening was done on eight individuals exposed to ionizing radiation. In all cases the frequency of S cells were slightly higher than in the normal control; however, their average age was also higher. One young adult with Hodgkin's disease in chronic remission after MOPP chemotherapy (plus radiation) was examined. The frequency of S cells was 2 x 10^{-7}.

In order to supply Lawrence Livermore Laboratory with monospecific fluorescent antibodies, the production of anti-Hb S has continued. The new technique developed at Lawrence Livermore Laboratory for staining of red blood cells in suspension has been tested with anti-Hb S FITC, and the results were very encouraging.

Antibodies to an expanding list of hemoglobin variants will be produced and used to investigate somatic cell mutation rates with respect to age and to investigate populations at mutagenic risk such as patients receiving cancer chemotherapy and those exposed to radiation. Screening programs would be able to identify populations exposed to mutagenic substances.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A system able to detect mutations in the genome of readily accessible somatic cells would allow estimation of the mutation rate in the human population. It would allow investigation of individuals and populations exposed to a mutagenic risk. There is no simple technique for screening at present. The antisera would also be useful in investigation of thalassemia and related genetic hemoglobin disorders.

PUBLICATIONS

Papayannopoulou, Th., Lim, G., McGuire, T. C., Ahern, V., Nute, P. E., and Stamatoyannopoulos, G.: Use of specific fluorescent antibody for the identification of hemoglobin C in erythrocytes. Am. J. Hematol. 2: 105-112, 1977.

Papayannopoulou, Th., Nute, P. E., Stamatoyannopoulos, G., and McGuire, T. C.: Hemoglobin ontogenesis: Test of the gene excision hypothesis. Science 197: 1215-1216, 1977.

Papayannopoulou, Th., Rosse, W., and Stamatoyannopoulos, G.: Fetal hemoglobin in paroxysmal nocturnal hemoglobinuria. Evidence for random derivation of the Hb F containing erythrocytes (F cells) from the PNH clone and from normal hemopoietic stem cell lines. Blood, in press.

Papayannopoulou, Th., Rosse, W., Stamatoyannopoulos, G., Chen, P., Adams, J.: Fetal hemoglobin in paroxysmal nocturnal hemoglobinuria (PNH): Evidence for derivation of Hb F-containing erythrocytes (F cells) from the PNH clone as well as from normal hemopoietic stem cell lines. Blood 52: 740-749, 1978.

Stamatoyannopoulos, G.: Possibilities for demonstrating point mutations in somatic cells, as illustrated by studies of mutant hemoglobins. In, Genetic Damage Caused by Environmental Agents, edited by K. Berg, Academic Press, New York, in press.

Stamatoyannopoulos, G.: Use of fluorescent anti-hemoglobin antibodies for the identification of normal and abnormal hemoglobins in single cells. In, Proceedings of the Conference on Prenatal Diagnosis of the Hemoglobinopathies, in press.

Papayannopoulou, Th., Vichinsky, E., Stamatoyannopoulos, G.: Foetal Hb production during acute erythroid expansion. I. Observations in patients with transient erythroblastopenia and post-phlebotomy. British Journal of Haematology, in press.

Hoffman, R., Papayannopoulou, Th., Landaw, S., Wasserman, L. R., DeMarsh, O. B., Chen, P., and Stamatoyannopoulos, G.: Fetal hemoglobin in polycythemia vera cellular distribution in fifty unselected patients. Blood, July, 1979.

LAWRENCE LIVERMORE LABORATORY - LIVERMORE, CALIFORNIA 94550
(Interagency Agreement Between NIEHS and Lawrence Livermore)

TITLE: Cytophysical Studies in Mutagenesis

CONTRACTOR'S PROJECT DIRECTOR: Mortimer L. Mendelsohn, Ph.D.

PROJECT OFFICER (NIEHS): Heinrich V. Malling, Ph.D.

COLLABORATING INSTITUTES: Department of Energy

DATE CONTRACT INITIATED: September 23, 1977

CURRENT ANNUAL LEVEL: $317,000

PROJECT DESCRIPTION

OBJECTIVES: Development of system for monitoring of the human population for mutations in vivo in single cells depends on the detection of a rare event in a single cell which is most effectively done by using flow systems. The scope of this contract is to develop such systems for red blood cells and sperm.

MAJOR FINDINGS AND PROPOSED COURSE: (1) The detection of somatic mutations in erythrocytes. At the present time it takes about one person-month to manually estimate the spontaneous mutation rate from HbA to HbS in a human. In order for this system to be practical the counting must be automized. This could be done through application of the flow system. A technique has been developed for labeling of red blood cells with fluorescent antibodies. The important step was to cross-link hemoglobin on the cytoplasmic side of the membrane. The flow system can now detect rare fluorescent ghosts in the midst of a large excess of unstained ghosts with total throughput rates of $\geq 10^6$ per second. Attempts will be made to increase the intensity of the AS cells and to improve the antibody preparations to eliminate precipitated protein as a source of false-positive signals. (2) Cytochemical fluorochromatic methods for dehydrogenase. It is possible to base a lot of mutation testing systems in somatic cell and in sperm in vivo on detection of variant hydrogenases. At the present time we use nitro blue tetrazolium to detect the dehydrogenases, and to find the variant cell we use a scanning microscope. This procedure, however, is very slow; and it would be a great step forward if we could use a cell sorter to sort the variant cells from the normal cells. All cell sorters are based, however, on detection of fluorescent signals. It is, therefore, important to develop dehydrogenase stains which can fluoresce. The synthesis of fluorescent indicator stains has taken two different routes; namely, (a) to couple an already fluorescent molecule to the tetrazolium or (b) to create a new tetrazolium which fluoresces after reduction instead of absorbing light in the visible area. (3) Distribution of DNA content in mice with various translocations. Detection of transmissible translocations is a very important tool for establishing risk of chemicals for the human population. At the present time the detection of translocations in mice after treatment of the parent requires a complex breeding program. During the meiosis the mouse, heterozygotic for translocation, will produce sperm with varying amounts of DNA. A question we ask here is, "Is that varying amount of DNA sufficient that we can detect a mouse, which is heterozygotic for translocation among normal ones, by comparing the variance of DNA in the sperm between the two mice?" The measurement of the DNA in sperm can be done on the flow system. A sufficient number of sperm can very easily be measured, and the variance can be compared. Since the mouse sperm is flat, there are severe problems in doing that. This problem has been solved by orientation of the sperm by means of hydrodynamic forces. A wedge-shaped tube positioned inside the standard sample tube causes formation of a thin ribbon-shaped sample core and oriented the sperm in one plane, resulting in a dramatic reduction of the variance in the level of fluorescence.

SIGNIFICANCE TO THE BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many compounds with widespread distribution in man's environment are known to be mutagenic or carcinogenic in laboratory test organisms. Man's constant contact with mutagens may pose more danger to the genetic health of the human population than does radiation. Nevertheless we do not have available today a monitoring system for the human population. Through collaboration of our in-house research, University of Washington, and this contract, we hope to develop such systems.

LABORATORY OF ENVIRONMENTAL BIOPHYSICS

LABORATORY OF ENVIRONMENTAL BIOPHYSICS

Summary Statement

The research program of the Laboratory of Environmental Biophysics is concerned with two main areas: the biological effects of physical factors present in our environment and the molecular interactions that occur between environmental agents and their biological targets (Molecular Biophysics). The physical factors under current investigation include non-ionizing radiation (microwaves), noise (including both auditory and non-auditory effects) and light. The Molecular Biophysics Program is mainly focussed on the use of sophisticated spectroscopic techniques to monitor the interaction of environmental agents with nucleic acids, membranes, proteins and microsomal systems.

Non-ionizing Radiation

The objectives of the non-ionizing radiation research program are as follows: 1) to develop microwave exposure systems for bioeffects research, 2) to develop and test techniques for measuring microwave energy absorption, 3) to determine the effect of microwaves on isolated nerve preparations, 4) to determine how 2450 MHz microwave radiation interacts with biological systems at all levels, 5) to study the effect of long-term exposure of experimental animals to 915 MHz and 2450 MHz microwave radiation on their CNS and behavior, 6) to ascertain the effect of 60 Hz fields from high voltage transmission lines on the CNS of mammals.

A waveguide system for exposing isolated neurons to 2450 MHz microwave radiation has been modified resulting in longer survival times and better temperature regulation of the nerves during exposure. Small thermistor probes have been used to measure deep colonic temperatures in mice during exposures to 2450 MHz microwave radiation. A system has also been developed and tested for exposing marine animals to different intensity levels simultaneously without significant perturbation of the microwave field by the location and orientation of the animals. A system for exposing localized areas or organs in animals has been assembled for use in physiological studies.

Research into the biological effects of microwaves at the cellular and organ level has continued. Experiments to determine the effect of continuous wave 2450 MHz microwave radiation on the vitality of the frog sciatic nerve have been completed. Distinct changes in the excitability and refractoriness of exposed nerves were seen for specific absorption rates of 10 mW/g and above. Ouabain, a specific inhibitor of active Na^+ and K^+ transport, caused both control and exposed nerves to run down faster and abolished the excitability difference between the two preparations. These findings suggest that the microwave induced loss of nerve excitability may be related to changes in ionic gradients that are normally maintained by active transport. Exposure of fertilized Japanese quail eggs to 2450 MHz microwave radiation at 5 mW/cm^2 did not cause any changes in either hatchability or development. Embryonic hearts removed after 8 days exposure in ovo to 5 mW/cm^2 also failed to show any significant morphological or enzyme changes. These experiments are now being repeated at 30 mW/cm^2. The mature quail from both exposure regimens will be subjected to immunological and biochemical testing. No changes were observed in the heart rate of embryonic quail exposed in ovo to continuous and pulsed 2450 MHz microwave radiation (0.3 mW/g to 30 mW/g specfic absorption rates) other than those which could be explained by temperature increases.

One of the most important areas of concern in non-ionizing radiation is the effect of long-term, low-level exposure. The long-term, low-level bioeffects of microwave exposure to 915 (918) MHz and 2450 MHz radiation are currently the subject of investigation by two contractors. The exposure of rats to 2450 MHz circularly polarized microwave radiation (5 mW/cm^2 average power density) for 10 hours per night for 16 weeks produced significant decrements in daily food and water intake although there was no difference in body mass between the control and exposed groups. No changes were observed in either the blood chemistry or the behavior of the irradiated animals. However, when pregnant rats were exposed to 500 μW/cm^2 2450 MHz circularly polarized guided waves for 20 hr/day during the first nineteen days of gestation, the following effects were observed in the offspring (1) increased neonatal deaths (2) decreased body weight on day 7 (3) later eye opening (4) inability to retain body heat (5) some growth acceleration (females only) (6) behavioral changes (females only). In other studies rats exposed to continuous 2450 MHz radiation (5 mW/cm^2 average power density) 8 hours/day for 16 weeks exhibited a significant depression of locomotor activity during exposure but no difference between exposures. There was no evidence of stress in the exposed group as measured by urinary 17-ketosteroid levels and the weights of adrenals, heart and liver although some slight alterations in blood chemistry were observed.

A third contractor is attempting to identify the peripheral and central receptors that are responsible for mediating the effects of microwave radiation on brain activity. In this study alterations in glucose consumption by auditory nuclei of rats during microwave exposure are being measured autoradiographically using [^{14}C] 2-deoxyglucose. Preliminary results with rats having one middle ear oblated have shown that pulsed microwave radiation can elicit a metabolic response in the auditory sytem by a mechanism which does not depend on conduction through the middle ear. In other experiments the responses of single fibers in the auditory nerve of cats exposed to pulsed microwave radiation have been recorded. While these studies have confirmed the hypothesis that a transduction of microwave energy induced mechanical disturbances occurs at the basilar membrane, they have also provided some evidence for a direct effect at the sensory hair cells of the cochlea.

Finally, the effect of 60 Hz electric fields on the mammalian central nervous system has been examined by a fourth contractor. In these studies rats were continuously exposed for 30 days to 60 Hz electric fields at 50, 500 and 1000 volts/meter. Two potential field dependent behavioral effects were found: rats exposed to 1000 V/m exhibited more active during the 0900-1000 period but were less active during the late night period. All groups of rats exhibited a general reduction in activity over the course of the study. This effect was field-independent and appeared to correlate with the size of each animal.

Noise

The overall objectives of the Noise Effects Workgroup are as follows: (1) to identify the physiological, biochemical and structural mechanisms which lead to cellular and neural damage associated with permanent hearing loss when activated by exposure to excessive noise or ototoxic agents. (2) To identify those environmental agents, drugs, etc. which potentiate hearing loss from noise exposure and to characterize the degree and extent to the additional hearing loss which occurs. (3) To study those specific non-auditory systems (endocrine, immunologic, physiologic, pharmacologic, teratogenic, cardiovascular) which may be affected by chronic noise exposure. (4) To identify by appropriate epidemiological methods those factors which are related to hearing loss and the non-auditory effects of noise.

At the present time most of the effort in noise effects research is concerned with the auditory effects with a smaller amount of time being devoted to the non-auditory effects. Areas under current investigation include (1) intracochlear ion transport differences between the normal and noise exposed animal, (2) quantitative and qualitative differences between damage caused by impact and steady state noise when equated for total energy content, (3) identification of the physiological mechanisms corresponding to complex signal analysis (speech) breakdown after slight noise trauma not predictable from simple signal pure tone tests, and (4) noise effects upon the fetus.

Considerable progress has been made towards understanding the factors which control ionic permeability of the endolymph-perilymph barrier in normal and noise exposed guinea pigs. In these animals the primary site of noise damage is at the level of the sensory hair cells of the organ of Corti. An apparent reduction in the permeability of the endolymph/perilymph barrier to potassium ions has been correlated with the suppression of hair cell responses during exposure to intermediate levels of noise. Prolonged exposure to high noise levels results in deterioration of the barrier and a concomitant fall in both the endocochlear potential and endolymph potassium concentrations. The combined effect of noise and ototoxic agents will be examined in future studies.

A prototype impact noise generator has been developed and its acute effect on guinea pig cochlear function compared with that of continuous noise exposure of equal energy. It is widely accepted that noises of equal energy produce similar cochlear damage. However our studies show that impact noise produces greater suppression of the cochlear microphonics while continuous broad band noise of equal energy gives rise to a greater suppression of the action potential. This effect will be studied more thoroughly after a new impact noise generator, that employs a pneumatic or hydraulic driving system instead of a solenoid, has been fabricated.

Studies on the coding of complex signals in the auditory system have continued. In this work speech-like inputs consisting of pseudorandom noise which has been amplitude modulated by sinusoidal signals are presented to the animal and the auditory nerve response in simultaneously recorded in the form of a cycle histogram. The cross-properties between output and known input, which represents the response characteristic of the peripheral auditory system, can then be determined. A computer system containing a high speed analog-to-digital converter, a high-speed clocked pulse counter and a low-pass sharp cutoff filtering system has been designed, built and tested. This system will be used to acquire baseline data on control animals prior to the testing of noise exposed animals.

Experiments to determine the effect of in utero noise exposure on the fetus have continued. The exposure of pregnant CF-1 mice to either semi-continuous high level noise (126 dBA jet engine noise) or unanticipated high intensity startling sounds resulted in embryo-lethality and decreased pregnancy maintenance. This effect did not appear to be related to elevation of plasma corticosterone levels. These studies will be repeated in guinea pigs since their audibility curve is very close to that of humans. Exposure of pregnant guinea pigs to textile mill noise (115 dB SPL) caused a significant deterioration in the hearing of the offspring as measured by the brainstem evoked responses technique.

A fiber optic lever capable of measuring displacements in the 10^{-2}-10^{2} Å range across 100 Hz - 20 kHz bandwidths has been designed and tested. This system will be optimized for measuring basilar membrane vibrations in normal and noise exposed animals. Finally some progress has been made in the development of a closed system electroacoustic transducer. This device will be used to present high level wide band minimum distortion sounds to the eardrums of guinea pigs.

The effect of protracted noise exposure on the cardiovascular function of primates is being examined via a contract. In these experiments, Rhesus monkeys are being exposed for periods of up to the one year to noise conditions resembling the community and workplace. Two animals have been exposed to term (8 months) however the loss of two consecutive control monkeys seriously hinders the interpretation of data at this time. The blood pressures of the two experimental monkeys after six months of chair restraint in the quiet were at the 50th percentile with respect to the indirect reference base. However the diastolic pressures had risen to the 99th percentile after six months of noise exposure. The results seem to implicate changes in the stroke volume rather than an increase in peripheral resistance. In the second phase of this study, stroke volume will be measured directly. In addition other physiological (dPdT, epicardial EKG, cardiac output) and biochemical (blood chemistry, plasma catecholamines and cortisol) parameters will alse be monitored.

Light

During the past year a new program concerned with the biological effects of artificial lighting and the interaction of light with chemical agents has been started. The beneficial effect of sunlight in the photoactivation of vitamin D precursors in the skin is well known. Cyclical changes in lighting also affect the maturation of gonads in both mammals and man. In addition to these effects sunlight elicits a number of undesirable side effects ranging from erythema ("sunburn") to skin cancer. More recently it has been suggested that artificial light sources, particular those which have energy spectra that are markedly different from sunlight may have undesirable side effects. The spectrum of fluorescent lighting, an almost ubiquitous light source found in both the home and work environment, is grossly distorted when compared to sunlight. For this reason, an experiment has begun to determine whether fluorescent lights have any hitherto unknown biological effects. For these studies, C_3H mice have been selected as the test species because they spontaneously develop mammary tumors and because previous studies have suggested that exposure to certain fluorescent lights decrease their lifespan. These studies have been ongoing for two months and no data is available as yet.

Another adverse effect of light results from its interaction with chemical agents in the skin. The chemical agent may be endogenous (e.g., protoporphyrin), a drug (e.g., sulfonamides, declomycin), topical agent (e.g., *p*-aminobenzoic acid in sunscreens) or an environmental agent (e.g., polycyclic aromatic hydrocarbons). The combined effect of light and these agents causes skin photosensitization which may take the form of either phototoxicity or photoallergy. While the initial step in all forms of photosensitivity must be the absorption of light by the chemical or its metabolite(s) the precise mechanism is unknown. We have therefore studied the effect of light on a series of aromatic compounds including two known photosensitizing agents, sulfanilamide and *p*-aminobenzoic acid. Results indicate that these compounds give rise to free radiclas on irradiation with light at wavelengths above 300 nm. While this finding suggests that free radicals may be responsible for the skin photosensitizing properties of these agents evidence will also be sought for other possible mechanisms including singlet oxygen formation and energy transfer to biologically important macromolecules.

Finally the photodegradation of polycyclic aromatic hydrocarbon absorbed onto solid phases is the subject of a grant that is currently being funded. In these studies, the photochemistry of particulate-absorved polycyclic aromatic hydrocarbons is being examined in a fluidized bed reactor. The supports has been made. A xenon arc lamp will be used for the exposure since it provides a wide spectrum of wavelengths similar to those formed in sunlight. This work has just begun and no results are currently available.

Molecular Biophysics

The main objective of the Molecular Biophysics Program is to understand at the molecular level the interaction of environmental agents with target biological systems including nucleic acids, proteins membranes and microsomal system. For these studies a number of highly sophisticated spectroscopic techniques, e.g., electron spin resonance and nuclear magentic resonance spectroscopy, fluorescence and absorption spectroscopy, circular dichroism and stopped flow spectrometry, are being employed.

Nucleic Acids. It is now widely recognized that many mutagenic agents exert their biological effects by modifying DNA. One of the purposes of this project is to synthesize base pair specific probes which can be used to monitor the interaction of mutagens with nucleic acids. The acridines, which bind strongly to DNA, are known to be mutagenic. Spin labeled analogs of 9-aminoacridine have therefore been synthesized and their binding to nucleic acids studied by means of a number of techniques. Two of these labels were found to have specificity for A-T and G-C base pairs respectively. With the aid of these labels it was possible to show that histone H_1 binds to A-T base pair rich regions of DNA in the minor groove. Three spin labeled analogs of actinomycin D have also been prepared. While they exhibited weaker bindings to DNA than the parent compound they were more active than actinomycin D against the P-388 tumor in mice. This finding may be related to the *in vivo* formation of superoxide.

The covalent binding of chemically reduced adriamycin and daunorubicin to DNA has also been examined. Results show that, under identical conditions, one adriamycin molecule is bound per 15 nucleotides whereas only one daunorubicin is bound per 140 nucleotides. These findings may explain why adriamycin induces more DNA damage than daunorubicin as evidenced by an increase in sister chromatide exchange.

Finally, it has found that several antitumor drugs induce membrane protein conformational changes in erythrocyte ghosts and mastocytoma cells. This finding suggests that the cytotoxic and mutagenic properties of these agents may involve membrane effects as well as interactions with nucleic acids.

Membranes. A large number of chemical agents are known to cause mast cell degranulation with the concomitant releaase of histamine, serotonin and other pharmacologically active compounds. While the initial event must involve the binding of these agents to the mast cell membrane, little is known about the molecular events that ultimately lead to degranulation. The interaction of compound 48/80 (a classical mast cell degranulating agent) with a number of membrane systems (erythrocyte ghosts, mastocytoma cells purified mast cells) has been studied in an attempt to elucidate the mechanism of histamine release. The fluidity of the mastocytoma cell membranes was found to be significantly greater than normal mast cells. Compound 48/80 did not affect the fluidity of either mastocytoma or mast cell membranes although it did increase the number of binding sites available for spin-labeled stearic acids. While other experiments did suggest that 48/80 induced a conformational change in the membrane proteins of erythrocyte ghosts the significance of this finding and its relevance to the effect of this agent on mast cells is unclear. With the aid of fluorescence microscopy it was possible to show that, at low concentrations 48/80 binds to sites in the cell membrane of mast cells and mastocytoma while at high concentrations binding also occurred to intracellular anionic sites (DNA in the mastocytoma cells and granules in mast cells). Finally, the effect of 2450 MHz microwave radiation at 5 mW/cm^2 and 100 mW/cm^2 incident power on 48/80 induced mast cell degranulation has also been investigated. The results showed that microwave radiation did not affect degranulation by 48/80. It has been suggested that microwaves may induce local temperature gradients across cell membranes. This appears unlikely in the case of mast cells since it is known that raising the temperature of the cells a few degrees above 37° causes an irreversible change in the membrane and results in a loss of sensitivity to compound 48/80.

Proteins. Work has continued on the interaction of heavy metal ions with human serum albumin (HSA). Cu^{2+} but not Co^{2+}, Ni^{2+}, Cd^{2+} and Zn^{2+} quenched the native tryptophan fluorescence of HSA. Stern-Vollmer plots indicated that fluorescence quenching occurs mainly via the energy transfer mechanism. The binding of Cu^{2+} to human, rabbit, canine and porcine albumin was measured with the aid of a copper ion sensitive electrode. HSA has two very high affinity Cu^{2+} binding sites ($K_a = 2 \times 10^6$ M^{-1}) and about seven lower affinity sites. Attempts to synthesize the N-terminal tripeptide of HSA (Asp-Ala-His-$NHCH_3$), which is thought to represent one of the high affinity sites for metal ions, have been unsuccessful. An alternate route will soon be attempted. Some success has been achieved in nuclear magnetic resonance studies of Cu^{2+} binding to HSA using ^{79}Br probe. These studies will be continued and expanded to include peptide fragments obtained by enzymatic digestion or chemical treatment of HSA.

Microsomal Systems. It is now generally accepted that the toxicity of many xenobiotics results from the generation of highly reactive chemical species (free radicals, singlet oxygen, superoxide) during metabolism. Nitroaromatic compounds are found in many forms in the environment including industrial solvents, antibacterial and antiseptic agents and poultry growth promoters. Previous work has shown that many of these compounds are metabolized by liver microsomes to form nitroanion free radicals which in the presence of oxygen give rise to the formation of superoxide. The microsomal metabolism of *cis* furylfuramide (AF-2), a nitrofuran antibacterial agent (food additive) and potent carcinogen, has been studied by means of electron spin resonance (ESR). Results show that *cis* AF-2 is isomerized to a 10:90 mixture of the *cis* - trans isomers through a nitroanion intermediate. This rearrangement apparently occurs before further "reductive" activation can take place. The free radical generated during the microsomal metabolism of carbon tetrachloride has been identified by spin trapping techniques as a lipid dienyl radical similar to that formed by the action of soybean lipoxygenase on linoleic acid. The superoxide radical has been detected in microsomal incubations containing adriamycin, daunorubicin or mitomycin C.

Personnel

The Laboratory has been reorganized into three separate Work Groups: Non-ionizing Radiation (Dr. Donald I. McRee, Head), Noise Bioeffects (Dr. Colin F. Chignell, Acting Head) and Molecular Biophysics (Dr. Colin F. Chignell, Head). Dr. Michael J. Galvin (Senior Staff Fellow) joined the Non-ionizing Radiation Workgroup in December, 1978.

Other Activities

Dr. Colin F. Chignell: Adjunct Professor of Pharmacology, Department of Pharmacology, School of Medicine, University of North Carolina at Chapel Hill, NIEHS representative, US-USSR Cooperative Program on Photobiology, member of American National Standards Institute Z-311 Committee on the Biological Effects of Non-ionizing Radiation; Managing Editor, Journal of Biochemical and Biophysical Methods; Editorial Board member: Molecular Pharmacology, Proceedings of the Society for Experimental Biology and Medicine, Environmental Health Perspectives, Chemico-Biological Interactions. Invited speaker at the Foundation Fund for Research in Psychiatry seminar in Clinical Pharmacology. Seminars at the Department of Pharmacology, School of Medicine, University of North Carolina, Chapel Hill and Department of Textile Chemistry, North Carolina

State University, Raleigh.

Dr. Reginald O. Cook: Member, Interagency Noise Effects Panel; member Committee on Hearing, Bioacoustics and Biomechanics of the National Research Council, National Academy of Science; member, Working Group 83 on Noise Effects

Dr. Michael J. Galvin: Participant, U.S.-U.S.S.R. workshop on the Biological Effects of Physical Factors, Seattle, Washington, June 11-15, 1979. Invited speaker at the Department of Physiology, Duke University, Durham, N.C., February 5, 1979. Elected a member of the American Physiological Society.

Dr. Teruzo Konishi: Member of Technical Committee, Physiological and Psychological Section, Acoustical Society of America.

Dr. Ronald P. Mason: Invited speaker by the Greater Washington DC ESR Group. Seminar at CIIT and participant in Special Problems in Toxicology Course, North Carolina State University, Raleigh, N.C. Elected a member of the American Society for Pharmacology and Experimental Therapeutics.

Dr. Donald I. McRee: Adjunct Professor, NCSU; Coordinator, US-USSR Cooperative Program on Health Effects of Non-ionizing Radiation; NIEHS representative on Inter-departmental Radiation Advisory Committee (IRAC) on Biological Effects of Non-ionizing Radiation; representative for DHEW on Interagency Advisory Committee on Electric Field Effects from High Voltage Transmission Lines (organized by DOE); representative of American National Standards Institute C-95 Committee on Safety Standards of Non-ionizing Radiation. Invited reviewer of WHO document "Environmental Health Criteria for Microwave and Radiofrequency Radiations", Geneva Switzerland; Invited to assist in rewriting WHO (European Office) report on "Microwave and Radiofrequency", appointed to National Research Council Committee on Biological Effects of Non-ionizing Radiation (reviewed report by PAVE PAWS panel); Invited speaker at N.Y. Academy of Medicine Symposium April 9-10, 1979; Invited speaker at the Bioelectromagnetic Symposium in Seattle, Washington, June 13-22, 1980; reviewer of NIOSH Draft "Criteria for Occupational Exposure to Radiofrequency and Microwave Radiation-Criteria for a Recommended Standard", Workshop organizer and chairman for US-USSR workshop on "Biological Effects of Microwaves and High Voltage Transmission Line Fields", June 11-15, 1979, Seattle, Washington.

Dr. Mary J. Ortner: Seminar at the Department of Pharmacology, School of Medicine University of North Carolina, Chapel Hill, North Carolina.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50005-05 LEB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of Noise and Ototoxic Agents on Energy Balance and Metabolism in Cochlea

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Teruzo Konishi	Medical Officer	LEB	NIEHS
	Alec N. Salt	Visiting Fellow	LEB	NIEHS
OTHER:	Philip E. Hamrick	Senior Scientist	RRP	NIEHS

COOPERATING UNITS (if any)

Research Resources Program

LAB/BRANCH

Laboratory of Environmental Biophysics

SECTION

Noise Effects Research Workgroup

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.3	PROFESSIONAL: 0.9	OTHER: 0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this project is to increase our understanding of the basic mechanisms of electrolyte movement across the endolymph-perilymph barrier in the guinea pig cochlea under normal conditions and under the influence of physical and chemical agents. The aim of the current project is (1) to reveal electrolyte movement (Na^+, K^+ and Cl^-) across the endolymph-perilymph barrier after exposure to intermediate levels of noise and (2) to correlate alteration of the ionic permeability of the cochlear partition with changes in sensitivity of the auditory system.

PROJECT DESCRIPTION

METHODS EMPLOYED: Healthy guinea pigs were exposed to broad band noise for seven days (16 hours on and 8 hours off per day). The overall intensities were 95, 105 or 115 dBA. After the period of noise exposure the cochlear potentials were recorded under anesthesia with pentobarbital sodium. The perilymphatic space was perfused with artifical perilymph containing ^{43}K, ^{22}Na or ^{36}Cl. Concentrations of Na$^+$ and K$^+$ in the perfusate, perilymph and endolymph were determined by a helium glow photometer and activites of these isotopes were measured using a gamma spectrometry system. Concentrations of Cl$^-$ in the fluid samples were measured by a microtitration method and the activities of ^{36}Cl were determined using a liquid scintillation counter. The degree of hearing loss resulting from exposure to noise was estimated by monitoring the brainstem evoked reponses from guinea pigs with chronically implanted electrodes at the vertex. The evoked responses were averaged with a computer.

MAJOR FINDINGS AND PROPOSED COURSE:

1. Brainstem responses

The Vth peak of the brainstem responses to unfiltered clicks measured at the end of the first exposure to noise at 95 dBA was markedly decreased in amplitude. Thereafter it remained little changed during the subsequent period. When the intensity of noise was increased to 105 dBA, responses of 2mV could be elicited only at the highest click intensity of 94 dB and remained unchanged during the entire period of exposure to noise. The average loss of sensitivity estimated by the Vth peak of the brain stem responses was about 40 and 70 dB immediately after 7 days exposure to noise at 95 and 105 dBA respectively.

2. Cochlear potentials

Following exposure to noise at 95 dBA, the mean ΔCM_{max} was 4.2 dB but there was no change in the cochlear microphonics (CM) sensitivity. An increase of noise level to 105 dBA resulted in suppression of the maximum CM output and in a loss of CM sensitivity of 7.5 dB and 13 dB respectively. The mean ΔCM_{max} and ΔCM_{sens} increased to 14 dB and 29 dB respectively when the noise level was raised to 115 dBA.

The action potential (AP) to 6 kHz tone bursts was more markedly suppressed by noise exposure than the CM. The mean loss of the AP, expressed by increment of sound level required to elicit 100 μV of AP was about 20 and 31 dB after noise exposure to 95 and 105 dBA respectively.

The mean values and standard deviation of the EP in guinea pigs exposed to noise at 95, 105 and 115 dBA were 85.3 ± 2.1 mV, 87.3 ± 4.9 mV and 91.7 ± 3.2 mV respectively. The EP values recorded in guinea pigs exposed to noise at 105 or 115 dBA were significantly greater than 84.0 ± 2.6 mV in control animals.

3. Electrolyte concentrations in the cochlear fluids

At intensities of 105 and 115 dBA, the mean K^+, Na^+ and Cl^- concentrations in the perilymph of noise exposed animals were not significantly different from those measured under control conditions. The mean concentrations of K^+ in the endolymph of guinea pig exposed to 105 or 115 dBA was 165.4 and 168.4 mM respectively and were significantly higher than control value (152.3mM). The concentrations of Cl^- in the endolymph were 138.1 mM and 133.8 mM in the guinea pigs exposed to noise at 105 and 115 dBA respectively.

On the other hand a marked reduction of Na^+ concentration was observed in noise exposed animals (0.7 mM and 0.4 mM in noise exposed animals at 105 and 115 dBA respectively).

4. Distribution of ^{43}K and ^{22}Na and ^{36}Cl in the cochlear fluids

Perfusate containing ^{43}K and ^{22}Na or ^{36}Cl was introduced into the scala tympani of the basal turn and the both scala vestibuli and tympani were perfused for periods ranging from 20 to 90 min in control and noise exposed animals. The normalized concentrations of ^{43}K, ^{22}Na and ^{36}Cl in the perilymph of the scala vestibuli with respect to their concentrations in the perilymph of the scala tympani did not show differences from control conditions. The concentrations of ^{43}K, ^{22}Na and ^{36}Cl in the endolymph increased as the duration of perfusion was prolonged in guinea pigs exposed for 7 days to noise at 105 and 115 dBA. In the steady state the concentration of a tracer in the endolymph can be expressed by

$$\frac{{}^*C_{endo}}{{}^*C_{peri}} = \frac{C_{endo}}{C_{peri}} \{1-\exp[-\lambda t]\}$$

where ${}^*C_{endo}$ and ${}^*C_{peri}$ are the concentrations of a labeled ion in the endolymph and perilymph respectively. C_{endo} and C_{peri}, the total concentrations of the ion in the endolymph and perilymph respectively, λ, rate constant, t, duration of perfusion. The rate constants for K^+ were 0.009 and 0.006 in guinea pigs exposed for 7 days to 105 and 115 dBA respectively. These values were significantly smaller than control value (0.013). The rate constants for Na^+ and Cl^- did not show significant difference between normal and noise exposed animals.

We plan to (1) perform studies of electrolyte movement across the endolymph-perilymph barrier under hyperthermic conditions and (2) study the combined effects of noise exposure at low body temperature.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The nature of the permeability of the endolymph-perilymph barrier to various electrolytes has been frequently hypothesized but the supporting data are rather limited. Present data suggest the possibility that electrolyte movement (especially K^+) across the cochlear partition is involved in the pathophysiological processes underlying noise induced hearing loss. These studies are a part of our efforts to increase our understanding of the disturbance of the inner ear under the influence of physical and chemical agents.

PUBLICATIONS

Konishi, T., Walsh, P.J. and Hamrick, P.E.: Ion transport in the cochlea of guinea pig. Acta Otolaryngol. 86: 22-34.

Konishi, T.and Hamrick, P.E.: Ion transport in the cochlea of guinea pig II chloride transport. Acta Otolaryngol. 86: 176-184.

Chen, B. and Konishi, T.: A simple current injector: Its application to coulomb titration of chloride. Int. Fed. For Med. and Engineering Computing. 16: 589-591.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50014-09 LEB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Microwave Exposure Systems and Microwave Dosimetry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Donald I. McRee	Research Physicist	LEB	NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Non-ionizing Radiation Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.1	PROFESSIONAL: 0.2	OTHER: 0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the objective of this project to develop microwave exposure systems for bioeffects research and to develop and test techniques for measuring energy absorption. The waveguide system for exposing isolated neurons to 2450 MHz microwave radiation has been modified so that longer survival times and constant temperatures of the nerves during exposure could be achieved. Small thermistor probes have been used to measure deep colonic temperatures in pregnant mice during exposures to 2450 MHz microwave radiation. Using the temperature profiles measured in the dead animals to calculate average absorption rates, the results agreed well with both calculated and measured whole body absorption reported in the literature. A system for exposing groups of marine animals to different intensity levels simultaneously without significant perturbation of the microwave field by the location and orientation of the animals has been developed and tested. A system to expose localized areas or organs in animals has been assembled for use in physiological studies.

PROJECT DESCRIPTION

METHODS EMPLOYED: The objectives of this project are to develop microwave exposure systems for biological material which have well-defined, field characteristics; and to develop dosimetric techniques for energy absorption. At present, it is difficult to measure accurately low level microwave fields and to determine the mechanisms by which these fields interact with matter. It is necessary, therefore, to conduct dosimetric studies so as to develop analytical interaction models. Microcalorimetry, thermistor and thermocouple detectors, and liquid crystals are some of the techniques which will be investigated. Other techniques such as implantable electric field probes to determine energy absorption will be evaluated as they become available.

MAJOR FINDINGS AND PROPOSED COURSE: It has been determined that for the exposure of small biological specimens such as cell systems and isolated neurons, waveguide exposure systems offer excellent characteristics. A waveguide system for exposing isolated neurons to 2450 MHz microwave radiation has been modified and improved. One modification permits the use of mineral oil in the neural stimulating and recording electrode modules so that longer survival times of the isolated nerves can be achieved. A second modification allows the circulation of saline through a constant temperature water bath into the waveguide exposure chamber. This modification enables us to maintain a constant temperature of both the control and exposed nerves during various power density levels of irradiation.

The system to expose small areas or organs of animals incorporates a small circular antenna. Due to the complex near field at the specimen, incident power density has little meaning, and therefore, the specific absorption rate in the tissue of interest must accurately be measured. A small thermistor probe 1mm in diameter with excellent accuracy and no interaction problems with the microwave field will be used to determine the specific absorption rates.

Waveguide exposure systems will continue to be fabricated, calibrated, and operated during the next fiscal year. The new systems will provide the Institute with the capability of exposing isolated neurons, cell systems and macromolecules to not only continuous wave, but other wave forms such as pulsed and frequency modulated waves. In addition, the new system will allow us to expose isolated mammalian nerves which are difficult to maintain in a viable state outside the animal body. The development of a system to measure the electrical properties, dielectric constant and conductivity, of biological material will continue.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The development of exposure systems with well-defined field characteristics and/or capability of accurate specific absorption measurements will enable the Institute to perform accurate quantitative studies on the effects of microwave radiation on biological systems at frequencies ranging from 1-10 GHz. Techniques to determine the amount of energy absorbed will provide the capability of evaluating the data in terms of thermal or specific microwave effects.

PUBLICATION

McRee, D.I. and Wachtel, H.: Long-term, low-level exposure of marine animals. Radio Science, (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50015-05

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of Microwaves on Neural Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Donald I. McRee	Research Physicist	LEB	NIEHS
Other:	Howard Wachtel	Consultant	LEB	Duke U.

COOPERATING UNITS (if any)

Duke University, Durham, North Carolina

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Non-ionizing Radiation Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.6	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to determine the effects of microwave radiation on neurological response. Isolated neurons such as the abdominal ganglion of the Aplysia, lobster ganglia, the sciatic nerves of frogs and the saphenous nerves of cats will be exposed to CW, pulsed, and modulated microwave radiation in the specific absorption rate range of 0 to 100 mW/g. Fatigue and recovery of the neurons under different modes of stimulation will be studied. The basic mechanisms of interaction will be investigated in cases where changes occur. Experiments to determine if continuous wave, 2450 MHz microwave radiation increases the fatigue rate or changes the vitality of the frog sciatic nerve were completed. The frog sciatic nerves were exposed in a waveguide to specific absorption rates of 100 W/kg, 50 W/kg, 20 W/kg. 10 W/kg, and 5 W/kg. The nerves were stimulated with twin pulses (separated by 5 ms interval) at a repetition rate of 50 pps in order to accelerate the rundown time. Distinct changes in the excitability and refractoriness of the exposed nerve were seen in comparison to the control nerves for specific absorption rates of 10 mW/g and above.

PHS-6040

PROJECT DESCRIPTION

METHODS EMPLOYED: Isolated neurons such as the abdominal ganglion of the *Aplysia*, lobster ganglia the sciatic nerve of the frog, and the saphenous nerve of cats will be exposed to CW, pulsed and modulated microwave radiation in the power density range of 0-100 mW/g. The effects of the radiation on nerve function and the mechanisms of interaction involved in any changes will be investigated.

MAJOR FINDINGS AND PROPOSED COURSE: A series of experiments to determine the effects of 2450 CW microwave radiation on the vitality of isolated frog nerves have been performed. The frog sciatic nerves were exposed in a waveguide to specific absorption rates (SAR) of 100 mW/g, 50 mW/g, 20 mW/g, 10 mW/g, and 5 mW/g. Circulating saline through the waveguide from a waterbath maintained the temperature at a constant level of 24°C for all exposure conditions. The nerves were stimulated with twin pulses (separated by 5 ms intervals) at a repetition rate of 50 pps in order to accelerate the rundown time. Distinct changes in the excitability and refractoriness of the exposed nerves were seen in comparison to control nerves for specific absorption rates of 10 mW/g and above. Several techniques have been used to determine the cause of the increase in rate of round down of the exposed nerve. Ouabain, which substantially blocks the active Na-K pump, has been used to treat the nerves before exposure. Both the control and exposed nerves fatigued at a faster rate than without ouabain, but there was no significant difference in the vitality of the two nerves under microwave exposure. This would lend support to the view that the relative loss of excitability in microwave exposed nerves is related to an interference with, or counteraction of the Na-K pump.

Another possible mechanism of interaction could be a change in ionic transport such that a Na-K imbalance would occur in the exposed nerve. Nonmyelinated, single axon lobster ganglia will be exposed and changes in transport monitored using labelled Na and K. Total sodium and potassium content, ATPase activity and spin-labelled membrane studies will also be performed. In addition to these experiments the sciatic nerve of frogs will be exposed to 2450 pulsed microwaves at average power densities in the same range as the continuous wave experiments. These studies will determine if pulsed microwaves are more or less effective in producing the change in vitality.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The potential health effects of microwave radiation in the environment is of interest to NIEHS. The neurological and behavioral effects reported in the literature illustrate the need for significant effort in this area. At present, the accepted safe level of exposure in the U.S. is 1000 times greater than the standard in the USSR. The Soviet standard is based on neurological and behavioral response to microwave radiation. This research on the effects of microwave on CNS is directed toward the mission of the Institute to determine the health effects of physical factors in the environment.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50016-07 LEB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Effect of Microwave Radiation on the Heart Rate of Embryonic Quail

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Philip E. Hamrick	Senior Scientist	RRP	NIEHS
Other:	Donald I. McRee	Research Physicist	LEB	NIEHS

COOPERATING UNITS (if any)

Research Resources Program

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Non-ionizing Radiation Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to determine if and how microwave radiation interacts with the embryonic quail heart or causes a change in heart rate. If a heart rate effect is found to exist, the change in heart rate as a function of absorbed microwave energy will be studied. Changes in heart rate due to temperature changes will be controlled.

PROJECT DESCRIPTION

METHODS EMPLOYED: Quail embryos in the egg are exposed to 2450 MHz CW and pulsed radiation in a temperature controlled waveguide. The temperature of the embryo is carefully monitored with a thermistor and the heart rate determined using carbon impregnated polymer for the electrodes.

MAJOR FINDINGS AND PROPOSED COURSE: Reports in the literature show that the heart is affected by exposure to microwave radiation. In our experiments embryonic quail were exposed *in ovo* to 2450 MHz microwave radiation and the heart rate monitored. No changes in heart rate have been observed that could not be explained by an increase in temperature on exposure to the microwave radiation. A variety of exposure intensities and conditions have been used. Exposure intensities giving specific absorption rates from 0.3 mW/g to 30 mW/g were employed. Both continuous wave and pulsed irradiation conditions were studied with the pulse repetition rate being varied from 10 pulses per second to 1000 pulses per second. The effect of microwave radiation in conjunction with the administration (over a range of concentrations) of the heart rate affecting drugs, propranolol and atropine was also studied. Since a significant change in heart rate due to microwave radiation was not found in any of these studies it is proposed that this project be discontinued for the present.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since it has been reported that microwave radiation affects the heart rate of perfused hearts, it is important to determine if the same effect exist *in vivo*. Our work suggest that if there are any microwave induced changes in the heart rate that these are compensated for by the presence of the intact central nervous system.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50017-06 LEB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Effects of 2450 MHz microwaves on Embryonic Development, Immunology, and Fertility

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Donald I. McRee	Research Physicst	LEB	NIEHS
	Michael J. Galvin	Senior Staff Fellow	LEB	NIEHS
Others:	J. P. Thaxton	Consultant	LEB	NCSU
	C. R.. Parkhurst	Consultant	LEB	NCSU

COOPERATING UNITS (if any)
Poultry Science Department, North Carolina State University

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Non-ionizing Radiation Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this research project are to study the effect of 2450 MHz microwave radiation on embryonic development, and immunology, biochemistry, and fertility of the mature quail. Fertilized Japanese quail eggs were exposed to 2450 MHz microwave radiation at incident power densities of 5 and 30 mW/cm^2 for the first 12 days of embryogenesis. The eggs were then transferred to a regular hatching incubator until hatch (17 days). Control eggs were treated in an identical manner. No differences were found in hatchability or development between the controls and 5 mW/cm^2 exposed quail. Data is not yet available on the 30 mW/cm^2 exposure. The quail were then placed in regular feeding and housing facilities and at 22 weeks immunologic and biochemical tests performed. No data is available on this aspect of the experiment. In some cases, embryonic quail were removed after 8 days microwave exposure and the hearts removed. No changes in electron microscopic appearance or tissue enzyme levels were evident in 5 mW/cm^2 exposed quail. The 30 mW/cm^2 data is not yet available.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The objectives of this project are to determine the effects of 2450 MHz CW microwave radiation on the embryological development of Japanese quail and the subsequent growth, reproduction, biochemistry and immunological response of the mature quail which had been exposed *in ovo* during the developmental period. Fertilized Japanese quail eggs were exposed to 2450 MHz CW microwave power at an incident power density of 5 or 30 mW/cm^2 for the first 12 days of embryogenesis. The effects of the exposure on hatchability and gross deformities were measured in the two day old quail. The quail were kept to maturity and growth rates, egg production, immunological response, reticuloendothelial system activity, and tissue and plasma enzymes were measured. In addition embryonic quail were removed after 8 day microwave exposure to either 5 or 30 mW/cm^2 microwave irradiation, the hearts dissected free, and examined using histological and biochemical techniques.

MAJOR FINDINGS AND PROPOSED COURSE: Exposure of the fertilized eggs to a power density of 5 mW/cm^2 and 30 mW/cm^2 for the first 12 days of embryogenesis were started. The eggs were subsequently transferred to a hatching incubator until hatch (17 days). Control eggs were treated exactly the same way. At hatch the quail were checked for deformities. No differences were found in the hatchability or development in the control and 5 mW/cm^2 exposed quail. No data is available on 30 mW/cm^2 exposure. The quail have been placed in regular feeding and housing facilities for 22-24 weeks and will not be exposed to any additional microwave radiation. At 22-24 weeks adult quail of both sexes will be examined as to weight gain, eggs laying (females), humoral and cellular immune responsiveness, reticuloendothelial system activity, and certain tissue and plasma enzyme levels. No data is avilable on these experiments . Embryonic quail hearts exposed to 5 and 30 mW/cm^2 radiation for 8 days were examined. Exposure to 5 mW/cm^2 for 8 days resulted in no changes in ultrastructural appearance of heart cells or alterations in tissue creatinine phosphokinase, or glutamic-oxaloxacetic acid transaminase activity. The 30 mW/cm^2 exposure group is now being done.

The proposed course of research will be to complete these studies on the effects of 5 or 30 mW/cm^2 microwave exposure.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The question of whether exposure to microwaves during embryonic development has any effect on the development process and function of the organism after maturity must be answered before microwave radiation exposusure can be considered safe. This project will assist the Institute in its mission of determining the health effects of physical factors in the environment.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50019-03 LEB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Effect of Ototoxic Insult on Coding of Complex Signals in the Auditory System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Teruzo Konishi	Medical Officer	LEB	NIEHS
	Reginald O. Cook	Acoustical Engineer	LEB	NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.35	.65	.70

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Results of behavioral and audiological tests with humans imply that ability to understand speech and other complex signals (particularly in a noisy listening situation) suffers deterioration from noise insult in excess of what could be inferred from pure tone thresholds measured in the quiet. These findings suggest that a complex interaction occurs between the various levels of the auditory nervous system and that functionally different receptors of the auditory end organ play a vital role in "sharpening" the sensory process. The objective of this study is to use the rapid signal analysis ability of minicomputers to determine the auditory response to speech and speech-like signals including pseudo-random noise under normal conditions and conditions of auditory fatigue. Auditory fatigue and recovery process of single nerve fibers to speech and speech-like stimuli will be studied. The requirement in high speed data throughput for nearly absolute phase matching of input acoustical and output physiological signals, and for accurately processing neural pulse trains necessitated the design and building of several unique complex peripheral devices. These are presently being debugged.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: In order to produce speech-like inputs most efficiently, pseudorandom noise which has been amplitude modulated by sinsusoidal signals is presented to the animal. The pseudorandom noise approximates the constantly changing frequencies of speech while the sinusoidal modulation simulates the varying intensities. When the sinusoidally modulated pseudorandom stimulus is presented to the animal the auditory nerve response is simultaneously recorded in the form of a cycle histogram, the cycle being locked to the period of pseudorandom noise. After summing the histogram many times to ensure statistical validity, cross-properties between the output and the known input, which represent the response characteristic of the peripheral auditory system, can be determined.

1. In order to obtain the temporal characteristics of the input noise a high-speed analog-to-digital converter (ADC) system capable of converting at speeds up to 40-50 KHz was necessary. Three channels (at roughly the same high speed) are needed for this and other planned experiments. Since no commerically available ADC system with a PDP-11 interface with sufficiently high speed existed, it was necessary to design and construct one around commercially available high-speed ADC modules and interfacing boards. This project was a cooperative venture involving Computer Engineering, BB, our electronic consultant, and LEB personnel.

2. For acquiring the histogram data, a special high-speed clocked pulse counter interfaced directly with the computer was designed by and built on a commerically available interface board by LEB personnel with design guidance from Computer Engineering, BB. As with the ADC, the special design was primarily necessitated by the 40-50 KHz data rate.

3. As an intrinsic adjunct to any high-speed ADC system, a low-pass sharp-cutoff filtering system must be provided in order to prevent aliasing of high frequencies. Phase matching requirements of such filtering systems when time dependent cross properties are to be measured far exceed those normally available filters. State of the art elliptic filters providing a higher maximum data rate and increased phase accuracy became available last year and were built into the system by LEB personnel.

MAJOR FINDINGS AND PROPOSED COURSE: This project has suffered from hardware interface problems due in part to the state of the art nature of the electronic interface mechanisms. As soon as these problems have been resolved, pilot projects involving normal animals to acquire baseline data and establish the resolution capability of the system will proceed; followed by subsequent testing of noise exposed animals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In recent years, it has become clear that the ability of the cochlear transducer to respond to pure tones is not an adequate indicator of its ability to respond to the natural complex fast changing stimuli present in the real world. Studies of the former are legion, studies in the latter area, particularly after ototoxic insult, are nearly nonexistent in spite of the fact that complex stimuli preceived in a noisy environment constitute the natural situation. We hope to obtain data which will begin to fill this void.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50020-03 LEB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Optical Fiber Motion Detector for Auditory System Measures

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Reginald O. Cook	Acoustical Engineer	LEB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.4	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Only a few techniques are capable of the resolution and frequency response necessary for auditory system motion measures, i.e., 10^{-2}-10^{2} Angstroms across 100 Hz - 20 kHz bandwidths. Previous theoretical and experimental efforts have led to determination of intrinsic performance limits and identification of the parameters relevant to opimization for given objectives. These analyses showed optic lever systems to be competitive with interferometric and optical heterodyneing techniques and better than capacitive probe techniques in resolution and an order of magnitude simpler and less expensive than either. Continuing refinement and explorative efforts have been focused in three areas: experimental determination of the optimum probe size linear range trade off and development of automatic in site calibration circuitry for umbo/ossicular chain measures; development and testing of high speed differentiation circuitry allowing correlation of mechanical impulses with accoustic emissions from imparted plate and other radiating structures creating occupational noise levels of hearing loss significance; and work on specialized probes for measurement of ossicular membrane vibration.

PROJECT DESCRIPTION

METHODS: Physics, electro optics, optics (fibers), electronic engineering computer modeling, noise measurements.

MAJOR FINDINGS AND PROPOSED COURSE: Basic fiber optic lever intrinsic performance limits: The relationships linking displacement detection limit (DDL), linearity, dynamic range, bandwidth, and working distance to fiber diameter, illumination irradiance and coupling angle, and electro-optical detection limit were determined for basic seven-fiber unit levers. Determination of reflected irradiance and subtended area of return fibers as functions of target distance revealed that both functions were nonlinear. However, the product of these functions, the lever displacement sensitivity, was found to deviate from linearity by less than 1% over target distance ranges equalling substantial fractions of fiber diameter at high injection angles and exceeding fiber diameter at low injection angles.

The optical detection limit was found to be shot noise limited when the photosensor consisted of appropriate commerically available photodiodes and op-amps connected in the transimpedance mode. The displacement detection limit was found to vary as the square root of the irradiance present on the target illuminating fibers. Linkage of this value to the power coupleable into fibers from selected sources, i.e., tungsten lamps and LED's, showed realizable DDL performance limits to lie in the 2×10^{-11} to 2×10^{-12} meters $/\sqrt{\text{Hz}}$ range.

The dynamic range was found to be limited by the op-amp saturation voltage to shot noise voltage ratio, a function of the square root of subtended power. At 10^{-3} watts, dynamic range was found to be approximately 130 dB/$\sqrt{\text{Hz}}$.

Frequency response was found to be linearly related to subtended power, extending well into the MHz region at high subtended power levels.

Working distance was found to be linearly proportional to fiber diameter and inversely proportional to the tangent of illumination coupling angle. Very large working distances can be obtained by design manipulation of these variables, by use of optic (lens) extender, or by separation of illuminate and return fibers.

Experimentally obtained measurements confirmed the validity of the analytically obtained relationships between the basic parameters.

Mechanical/acoustical shock transducer: Displacement, velocity, and acceleration amplitude and frequency detection limits were analytically determined and electronic circuitry designed and built. Measurements of the acceleration, velocity, and displacement waveforms from an impactively excited plate made by a miniature shock accelerometer and a fiber lever clearly showed the superiority of the latter. Experimental determination of whether actual performance limits match predicted performance limits will be undertaken as soon as an LED driver capable of the necessary dynamic range and frequency response needed to test the shock optimized lever is completed.

Auditory System Optimization: The small size of the termination of the ossicular chain (umbo) in the center of the tympanic membrane coupled with the relatively large "DC" movements imposed on the ossiclar chain by the middle ear muscles pose serious motion measurement problems. Since working distance and linear range are directly proportional to fiber diameter, use of fibers which are too small allows the spontaneous "DC" motions to carry the umbo beyond the linear range of the lever, while use of oversize fibers results in illumination of areas bigger than the umbo, introducing measurement errors. After analytical determination that the optimum fiber diameter was on the order of 125 microns, fibers of 100, 125, and 200 diameter were ordered and probes fabricated. Experimental verification of the optimum probe will be undertaken along with shakedown of electronic circuitry built to allow automatic in situ calibration, an important advantage since the ossicular chain is constantly perturbed by middle ear muscle movements.

Fiber Lever Optimized for Basilar Membrane Vibrations: Little effort has been devoted to this effort during the past year due to the time required to complete other projects. Several fiber manufacturers who say they can produce the 25-40µ fibers and accomplish the necessary 90°, 100µ bend ratios have been located. Several such bundles will be purchased in the near future for evaluation

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Shock and vibration transducer: Reduction of harmful noise emissions from machinery and other rotating sources can best be avoided at the design stage provided theory on which predictions of the noise emissions resulting from mechanical shock is available. Frequency response of accelerometers used in shock and vibration measurements is usually limited by linearity considerations to one-third of the resonant frequency of the accelerometer. In addition, for highly sensitive measurements, the added mass of accelerometer may alter the characteristics of the surface motion. And finally, to obtain displacement waveform measurements, the signal obtained through an accelerometer must be integrated twice with great loss of frequency response and dynamic range. The use of noncontacting fiber optic levers offers an attractive alternative in some shock and vibration problems, particularly where knowledge of displacement waveform characteristics is desirable.

Optimized Ossicular Chain and Basilar Membrane Auditory Motion Transducer: A convenient means for making audio frequency displacement measures of the ossicular chain at displacement levels (1-100 Angstrom) corresponding to normal sound levels (40-80 dB SPL) has long been needed in the study of auditory distortion and hearing loss mechanisms. Reliable measures of the motion of the basilar membrane would resolve present uncertainties concerning non-linear plateau effects at high intensities and frequency resolution from high and low frequency slopes of the maximas; these uncertainties block confirmation of hearing theories at the intracochlear mechanical level.

In addition to these specific applications, optic levers have been utilized to measure the dynamic force exerted by muscle myofibrils, as the basis for ultra-sensitive pressure transducers for cardiovascular and other clinical purposes. Although the non-contacting characteristic of optic levers uniquely qualify them for biological (and many mechanical applications), application of the technique has previously suffered from lack of a theoretical basis from which design optimization could proceed, and from the relatively short working distances and low detection limits associated with the commericially available models. Since the theoretical relationships have been developed for both laser and Lambertian sources and confirmed by measurement, it is now possible to accurately predicted the ultimate resolution for given fibers, bundle configurations and illumination sources.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50022-03 LEB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Development of Impact Noise Generator for Hearing Loss Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Reginald O. Cook	Acoustical Engineer	LEB	NEIHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Environmental Biophysics

SECTION

Noise Effects Research Workgroup

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.40	0.30	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to refine a previously constructed prototype impact noise generator whose peak level, rise time, and decay can be experimentally manipulated so that the effects of these parameters on hearing loss can be studied in laboratory animals. Although the initial impact noise generator demonstrated that the desired sound pressure level and waveshapes could be produced the mechanical failure rate was excessive. Nevertheless it was possible to show that the response of the cochlear microphonics and action potential differed significantly between impact and continous noise of equal energy, the mechanical failure rate was excessive. A pneumatic or hydraulic driving system was deemed necessary to withstand the hundreds of thousands of impacts necessary in a chronic exposure experiment. Arrangements are being made for the design and fabrication of a pneumatic driver.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: In order to simulate exposure to impactive noise in industrial situations various impact noise generation mechanisms were investigated analytically and experimentally to determine the vital characteristics of the radiated sound pulses, e.g. rise time, frequency content, energy level, peak pressure level, repetition rate, etc. Based on these findings, a prototype impact sound generator was designed for use in laboratory noise exposure studies. The motive source chosen for this initial generator was a very large solenoid, which allowed impact repetition rate manipulations. The impact of the solenoid was actuated by a specially designed electronic circuit to control duration of each impact, applied voltage, which in turn determined the speed, and finally the repetition rate of the impacts.

MAJOR FINDINGS AND PROPOSED COURSE: The four basic impact sound generation mechanisms were found to be air ejection from between the colliding surfaces, radiation due to acceleration of the impacting rigid bodies, radiation due to rapid surface deformations caused by collisions, and pseudo-steady state radiation (ringing) of the impacted object. Except under ideal conditions, the sound radiation from the impact of two or more solid bodies is a result of a combination of several of these mechanisms. The sound pressure levels produced by the designed prototype "impact heads" reach 140 dB which is well in the range necessary to simulate industrial exposures of 120-160 dB peak. The initial generator was an acoustical success but its mechanical failure rate was excessive.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The fact that it is not possible to reproduce impact sounds using conventional audio equipment has resulted in few if any well controlled laboratory studies of hearing damage from a type of noise which pervades industry and society. There is strong suggestive evidence that high intensity, short rise time peaks, rather than steady state levels, are the major contributor to hearing loss. Because the temporal summing characteristics of the human auditory system operate over a longer time span than the duration of impact sounds, perceived loudness is not proportional to peak level or to peripheral organ damage possibilities. This effort should make refined studies of hearing loss/impact noise parameters possible.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50026-02 LEB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Development of Closed System Electroacoustic Transducer for Noise Research

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Reginald O. Cook	Acoustical Engineer	LEB	NIEHS
Others:	Teruzo Konishi	Medical Officer	LEB	NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina

TOTAL MANYEARS: .25	PROFESSIONAL: .20	OTHER: .05

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

For some time, the ability to perform experiments requiring the presentation of high level, wide band, minimum distortion sounds to the eardrum of guinea pigs has been unduly restricted by presently available small electroacoustical transducers suitable for earbar interface. As a result, high level sounds have had to be produced from loudspeakers mounted some distance from the ear (field stimulation). As a result, distortion, maximum achievable level and accuracy of measurement suffer. The availability of electroacoustic transducers operated into closed systems (earbar or ear canal) and capable of producing very high levels would allow one time acute exposures at levels sufficient to cause immediate auditory damage resulting in significant time savings. In addition, the pseudorandom noise which will serve as stimulus in some experiments, is characterized by a line spectrum. Prevention of distortion in line spectrums is particularly important. There have been reports in the high fidelity literature of earphone transducers having many of the desirable characteristics, so an attempt will be made to have such a device optimized for closed system applications.

PROJECT DESCRIPTION

METHODS EMPLOYED: Very sophisticated level, frequency and distortion tests of any electroacoustics transducer can be performed in our laboratory. Devices will be built to our specifications by others.

MAJOR FINDINGS AND PROPOSED COURSE: Small commercially available electroacoustic transducers are optimized to operate into an acoustic impedance simulating that of the human ear canal and pinna, (6 ml). A change in this volume of as much as 10% causes significant frequency response and distortion changes. The acoustically compliant volume of conventional ear bars and guinea pig ears is about 0.5 ml. Mass and resistive loading are negligible. Manufacturers will be approached in an effort to get an optimized prototype built to our specifications. Little was accomplished during the past year on this project.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Sound sources with which precise control (i.e., no distortion) of acoustic waveforms at the ear, across wide band width and amplitude can be produced. It is believed that current electroacoustic transducer technology can be exploited to achieve advances in noise exposure research.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50027-02 LEB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Prenatal Exposure to High Noise Levels on Auditory Thresholds in Guinea Pigs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Reginald O. Cook	Acoustical Engineer	LEB	NIEHS
Others:	Teruzo Konishi	Medical Officer	LEB	NIEHS
	Alec Salt	Visiting Fellow	LEB	NIEHS

COOPERATING UNITS (if any)

Laboratory of Environmental Toxicology

LAB/BRANCH

Laboratory of Environmental Biophysics

SECTION

Noise Effects Research Workgroup

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina

TOTAL MANYEARS: .8	PROFESSIONAL: .5	OTHER: .3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pregnant guinea pigs were exposed to tape recorded textile mill (weaving room) noise at 115 dB Sound Pressure Level, for 8 hours/day, 5 days week. Exposure generally began at the third trimester of pregnancy, but varied due to discrepanies between the estimated breeding date and the actual breeding date as determined by delivery. The hearing levels of offspring were measured and compared with that of offspring from otherwise identical mothers. Brainstem evoked responses recorded from chronically implanted scalp electrodes showed deterioration in the hearing of the offspring from exposed mothers. These differences are currently being analyzed to determine statistical significance.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Pregnant guinea pigs were exposed to tape recorded weaving room noise in an acoustically shielded chamber, 8 hours per day, 5 days per week. The hearing levels of offspring were tested on either 2 or 3 sequential occasions by the noninvasive sound evoked brain-stem response (BSER) and selected animals from each litter were tested by measurement of the N_1 action potential and by cochlear microphonics before sacrifice. Temporal bones (cochleas) were saved for subsequent histological analysis. BSER techniques involve computer averages of thousands fo stimuli/responses rapidly.

MAJOR FINDINGS AND PROPOSED COURSE: Hearing levels of the offspring from guinea pigs exposed to high level 115 dB (150 Hz 8 kHz) textile mill noise during latter third of pregnancy were found to differ consistently from the hearing levels of offspring from non-exposed but otherwise similar females. Hearing was measured in all animals by brainstem evoked responses and additionally by the cochlear microphonics and N_1 action potential (from one animal per litter). Preliminary results are similar to those of Daniel, a Polish researcher, who found hearing levels of the offspring from exposed mothers to be worse than controls. There were some, perhaps significant, differences in the two studies: Pregnant females were exposed full term in the Polish study and hearing was measured by a rather crude behavioral technique.

This project afforded the opportunity to compare computer analyses of averaged BSER data in several ways: RMS, 5th peak amplitude, 5th peak latency, and entire waveform time lag correlation. The latter in particular is much easier to perform, and further studies will be undertaken to verify the possibility of replacing 5th peak latency with correlation lag. This would provide significant advantages in small animal testing, because latency differences are very small relative to humans.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Because of the rapid movement of females of childbearing age into occupations traditionally dominated by men, some of them very noisy, increasing concern has been voiced regarding possible general teratogenic and specific auditory risks to fetuses of mothers so exposed. One indication of this concern was the request made by the Air Force to the National Academy of Sciences Force that they convene a scientific panel to assess that risk. The panel has not yet reported its findings.

In 1977 Bock and Sanders presented compelling evidence for the existence of a period of enhanced susceptibility to auditory system damage from noise insult in hamsters. The inception of this critical period corresponded to the beginning of structural maturation of the auditory system (approximately day 20 after birth in hamster) and continued for about 25-30 days. Because of the very short fetal stage in hamsters (18 days), the corresponding stage occurs prenatally in humans. Previously Falk, Cook and Haseman have presented histological, evidence for enhanced auditory damage susceptibility of very young versus older guinea pigs. Recently Daniel, in a 1976 study in Poland, reported that the offspring of pregnant guinea pigs exposed to weaving mill noise (105 dBA) had higher threshold levels (poorer hearing) than the offspring of mothers not so exposed. Because of the comparatively long fetal period of guinea pigs (68 days), the period corresponding to auditory system structural maturation also occurs prenatally. The results of this study show that late pregnancy high level exposure to noise has little if any effect on the sensitivity of the hearing organs, of guinea pigs, which is contrary to suggestive evidence outlined above.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50028-02 LEB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
The Effects of Noise and Drugs on the Electrochemistry of the Cochlea

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Teruzo Konishi	Medical Officer	LEB	NIEHS
	Alec N. Salt	Visiting Fellow	LEB	NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.5	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aim of the project is to find physiological correlates to account for the loss of cochlear sensitivity during the initial stages of noise overexposure. Anesthetised guinea pigs are exposed to broad band or octave band continuous noise. During exposure, cochlear function is assessed by the measurement of endocochlear potential, tone-induced cochlear responses and the Na^+, K^+ and Cl- activities of endolymph and perilymph. Results indicate that the primary site of noise damage is at the level of the sensory hair cells of the organ of Corti. An apparent reduction of the permeability of the endolymph/perilymph barrier to potassium ions is correlated with the suppression of hair cell responses during exposure to intermediate levels of noise. After prolonged exposure to high noise levels, the endocochlear potential and endolymph potassium concentration fall presumably as a result of deterioration of the endolymph/perilymph barrier.

PROJECT DESCRIPTION

METHODS EMPLOYED: The activities of K^+, Na^+ or Cl^- were monitored continuously in the endolymph or perilymph of the anesthetized guinea pig with double barreled, liquid membrane ion sensitive microelectrodes. The external tip diameter of electrodes was 1-2μ and the electrical impedance was between 10^9 and 10^{10} Ω. Electrodes were inserted into scala media or scala tympani through a small fenestra in the bony wall of the cochlea. The endocochlear potential was recorded from the ion-insensitive barrel of the electrode. Tone induced cochlear potentials evoked by 6 kHz tone bursts were recorded from differential electrodes in the perilymphatic scalae of the basal turn. The endocochlear potential (EP) and electrolyte activities were monitored during repeated exposure to noise for 20 min periods. Cochlear sensitivity to test tones was measured between successive exposure periods.

MAJOR FINDINGS AND PROPOSED COURSE: When the cochlea was exposed to 95 dBA or 105 dBA broad band noise the action potential (AP) sensitivity was suppressed, requiring a mean increase of 10.7 dB and 35.0 dB respectively in the intensity of the test tone required to elicit 100 μV response (AP_{sens}). The cochlear microphonics (CM) were only slightly changed, the mean test tone increase required to maintain 100 μV output (CM_{sens}) being 0 and 2.3 dB for 95 and 105 dBA broad band noise respectively. The EP showed a negative deflection at the noise onset (negative summating potential) which was slightly suppressed during the course of exposure but there were no major changes of the EP value measured under quiet conditions. There were also no systematic changes of endolymph or perilymph potassium concentrations at these noise levels.

At the 115 dBA noise level AP_{sens} was reduced by 35.4 dB and CM_{sens} was reduced by 22.5 dB. CM suppression was accompanied by an increase in the endocochlear potential of 5.1 mV and an increase of the endolymph potassium concentration $[K^+_{endo}]$ of 2.5 mM. Repeated exposure of 20 min periods of at this intensity only resulted in slight further suppression of the tone induced responses and no further increase in EP. $[K^+_{endo}]$ repeatedly increased during exposure periods and decreased during quiet periods.

Cochleas pre-treated with ototoxic antibiotic kanamycin were also subjected to broad band noise exposure at 115 dBA. Prior to noise exposure these preparations showed an almost total abolition of the tone induced responses, indicating severe hair cell degeneration. In contrast the EP and $[K^+_{endo}]$ were within the normal range indicating the stria vascularis was functionally normal. During 115 dBA noise exposure the preparations exhibited no increase in EP or $[K^+_{endo}]$, suggesting that the changes of EP and $[K^+_{endo}]$ measured in the normal cochlea arise from changes at the air cells and not from changes in the active ion transporting activity of stria vascularis, as had previously been hypothesised. We have therefore proposed that the increases of EP and $[K^+_{endo}]$ during 115 dBA noise exposure arise from a reduction in the potassium permeability of the organ of Corti.

When the cochlea was exposed to low frequency octave band noise at 125 dB SPL the CM and AP were rapidly suppressed, ΔCM_{sens} and ΔAP_{sens} falling by 32.2 and 41.2 dB respectively during the first exposure period. The EP initially increased above the pre-exposure level but after 2 or 3 exposure periods both EP and $[K^+_{endo}]$ began to slowly decrease in value, probably due to the direct mechanical disruption of the reticular lamina and hair cells.

The project will be pursued in the following directions.

1. The recent availability of liquid ion exchangers sensitive to sodium has enabled the present technique to be applied to the measurement of sodium changes in the endolymph and perilymph. The possible disturbance of sodium distribution during noise exposure will therefore be examined.

2. The possibility that drugs known to affect membrane permeabilities (e.g. amiloride) or the vascular system (e.g. salycylic acid, epinephrine) may alter the course of noise induced hearing loss will be examined.

3. The technique for manufacturing ion selective electrodes has now been improved, enabling the production of reliable electrodes with sub-micron tip diameters. We hope to use these electrodes to record from single cochlear hair cells in the normal and noise exposed cochlea. The technique will involve dye-marking each cell from which a recording is taken in order to verify the recording site and cell type. This would provide valuable information about the physiological changes within the hair cells during noise exposure.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Although the characteristics of the hearing deficit produced by noise exposure have been well documented, the mechanism by which noise produces the deficit has not yet been established. Our results demonstrate that the primary changes resulting from continuous noise exposure may be permeability changes of the endolymph/perilymph barrier.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50029-02 LEB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Acridine Spin Labels as Probes for Nucleic Acids and Their Protein Complexes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Birandra K. Sinha	Senior Staff Fellow	LEB	NIEHS
Others:	Colin F. Chignell	Chief	LEB	NIEHS

COOPERATING UNITS (if any)

NONE

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.45	PROFESSIONAL: 0.45	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

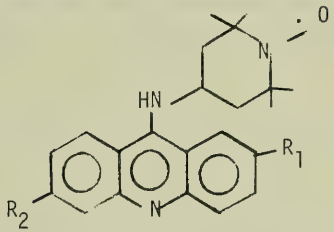
SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to design spin label probes which can be used to study the binding of mutagens to nucleic acids. Since acridines are mutagenic and bind strongly to nucleic acids, we have designed and prepared acridine spin labels containing the stable nitroxide free radical. The binding of these acridine spin labels to nucleic acids has been studied by electron spin resonance. These spin label derivatives bind to nucleic acids by intercalation and have high base affinity. However, this affinity is not absolute. We have used these labels in studying the binding of histone H_1 to DNA. Our studies suggest that H_1 binds to the A-T-rich bases of DNA in the major groove.

PHS-6040
(Rev. 10-76)

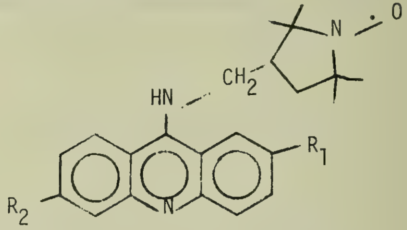
PROJECT DESCRIPTION

METHODS EMPLOYED: The nature of the interaction between aminoacridines and nucleic acids is of considerable interest because of the mutagenicity of these compounds and their reported antitumor activity. The biological properties of the acridines are believed to arise either from their intercalation into DNA (or RNA) or from their association with phosphate groups on the outside of the double helix of nucleic acids. We have therefore synthesized aminoacridine spin labels I-VII and studied their interaction with RNA and DNA by means of electron spin resonance and other spectroscopic techniques.



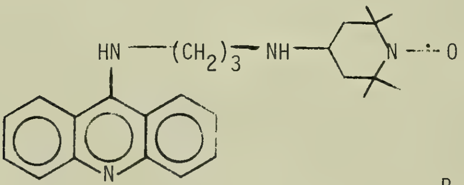
R_1 = OCH_3, R_2 = Cl = 1

R_1 = R_2 = H = II

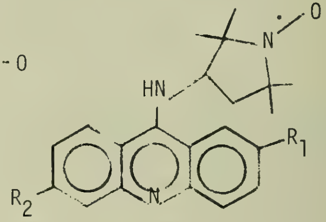


when R_1 = OCH_3, R_2 = Cl = III

R_1 = R_2 = H = IV



V



when R_1 = OCH_3, R_2 = Cl = VI

R_1 = R_2 = H = VII

Acridine spin labels were synthesized from their corresponding 9-chloroacridines and the respective spin labeled amines. Electron spin resonance (ESR) spectra were recorded with a Varian E-4 spectrometer operating at 9.5 GHz. Samples were introduced into the cavity in quartz micro flat cells. The temperature of the samples was maintained with a Varian variable temperature accessory and was measured with a Yellow Springs Instrument Company telethermometer. A Cary 15 spectrophotometer equipped with a water jacket was used for the T_m determinations. Sedimentation velocity measurements were made with a Beckman Spinco Model E analytical ultracentrifuge.

MAJOR FINDINGS AND PROPOSED COURSE: The ESR spectrum of acridine spin label I bound to DNA was broad and asymmetric with a maximal hyperfine splitting ($2T_m$) of 58.7 G. Neither heat denaturation of the DNA nor the addition of salt (0.1 M NaCl) altered the $2T_{\parallel}$ value of bound label I (Table 1). The $2T_m$ value of acridine spin label II bound to DNA was 55.5 G. The addition of 0.1 NaCl to the DNA-II complex did not significantly alter the ESR spectrum of the probe (Table 1).

TABLE 1

THE MAXIMAL HYPERFINE SPLITTINGS ($2T_m$) OF ACRIDINE SPIN LABELS BOUND TO CALF THYMUS DNA

Sample	NaCl (0.1 M)	$2T_{\parallel}$ (G)
DNA + I	-	58.7
DNA (heat denatured) + I	-	59.0
DNA + I	+	59.0
DNA + II	-	55.5
DNA + II	+	55.0
DNA + III	-	66.5
DNA + III	+	67.0
DNA + IV	-	57.0
DNA + IV	+	57.0
DNA + VI	-	56.0
DNA + VI	+	56.0
DNA + VII	-	61.5
DNA + VII	+	61.0

In the absence of spin label, DNA was found to have a sedimentation coefficient of 11.2 S while on the addition of spin label I, the sedimentation coefficient decreased to 8.92S. This observation suggests that label I is indeed intercalated into the DNA. The effect of the acridine spin labels on the melting temperature (T_m) of DNA was studied by means of absorption spectroscopy and electron spin resonance. The T_m value for DNA alone was found to be 66.5° as determined by the increase in the optical density of the solution at 260 nm. In the presence of spin label I, the melting temperature of DNA was raised to 70°. It was also found possible to measure the T_m value for complexes between the spin labels I and II and DNA by monitoring the concentration of free spin label in solution as function of temperature. The T_m value of a complex between DNA and I was found to be 69° by this technique. The complex between DNA and II exhibited T_m values of 76° by the spectrophotometric assay and 78° by the ESR method.

From the ESR spectra of complexes between labels I and II and DNA, it is apparent that the piperidine ring that bears the nitroxide has greater mobility in the complex between DNA and II. This suggests that there is a fundamental difference in the way that these two spin labels interact with DNA.

In order ascertain the nature of the DNA binding sites for I and II, studies were carried out with two synthetic polynucleotides, polydA-polydT and polydG-polydC (Table 2). These data indicate that

TABLE 2

THE HYPERFINE SPLITTINGS ($2T_m$) OF I AND II BOUND TO DNA,

PolydA-dT AND PolydG-dC

Label	Nucleic Acid	$2T_m$ (Gauss)
I	DNA	59.0
I	PolydA-dT	62.5
I	PolydG-dC	59.0
II	DNA	55.0
II	PolydA-dT	55.5
II	PolydG-dC	62.5

label I binds preferentially to the G-C regions in DNA while Label II binds to the A-T-rich regions of DNA. In order to characterize further the binding of I and II to DNA, displacement studies were carried out with daunorubicin, an antitumor drug, known to bind DNA by intercalation. Furthermore, it been shown that daunorubicin has a high affinity for A-T-rich regions of the DNA. When daunorubicin was added to complexes formed between I and DNA in 5mM phosphate, 30% of the bound I was displaced. In constrast, the addition of daunorubicin to DNA-II complexes under similar conditions caused a 70% displacement of bound II from DNA. In the presence of 0.1 M NaCl, which decreases the binding of I and II at the phosphate groups of DNA, daunorubicin displaced only 12% of the bound I from DNA. However, the addition of daunorubicin caused a much larger displacement (46%) of the bound II from DNA. A larger displacement of II from DNA coupled with rather small displacement of bound I by daunorubicin suggest that II occupies similar binding sites as daunorubicin in DNA; namely the A-T-rich regions of DNA.

Nucleohistones, complexes of DNA and histones, are thought to be involved in genetic regulation. The mechanism of this control may involve changes in the interactions and conformations of nucleohistones. The molecular structure of nucleohistone is not precisely known, however, considerable progress in this area has been made since the isolation of repetitive subunits called nucleosomes of nu-bodies. Histone H_1, composed about 25% lysine residue is not part of the nuclesome proper but is bound to the linker regions between adjacent particles.

When histone H_1 was added to a complex formed between I and DNA at a low P/D ratio some of the bound I was displaced into the solution. In the presence of 0.1 M NaCl, which precludes ionic binding at phosphate groups, addition of H_1 had little or no effect on bound I. In contrast, H_1 caused a significant displacement of bound II from DNA in the presence of NaCl. At a high P/D ratio, where I and II are primarily bound by intercalation only, addition of H_1 to DNA did not displace either I or II. The addition of NaCl did not alter this effect.

These findings suggest that II and H_1 may be occupying the same binding sites on DNA. Since our finding also suggests that daunorubicin and II occupy the same binding sites, A-T rich bases, and since daunorubicin is known to be intercalated through the major groove of the DNA helix, we conclude that H_1 must also bind to the A-T rich regions of the DNA. In addition, the lysin groups of H_1 interact with the functional group of the major groove of the DNA.

Future plans include studies on the binding of repressor proteins to DNA using these spin label acidines and a detailed analysis of the DNA conformations upon binding of histones to DNA.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Acridines are of interest for two reasons; firstly, because of their biological and pharmacological effects and secondly, because they may be useful tools for probing the conformation of nucleic acids in biologically important complexes such as chromatin, DNA-repressor proteins, etc. In both areas, it is expected that these acridines spin labels will provide useful information, not only on the pharmacological and toxicological effects of acridines in biological systems, but also in the area of gene transcription.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50030-02 LEB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Spin Labeled Actinomycin-D Analogs as Base Specific Probes for Nucleic Acids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Birandra K. Sinha	Senior Staff Fellow	LEB	NIEHS
Others:	Colin F. Chignell	Chief	LEB	NIEHS
	R.L. Cysyk	Chief, Drug Metabolism	NCI	NIH

COOPERATING UNITS (if any)

Laboratory of Chemical Pharmacology, NCI, NIH

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.45	PROFESSIONAL: 0.45	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to prepare base specific spin label probes to study binding of chemical carcinogens to nucleic acids. For this purpose, we have prepared spin labeled actinomycin-D to probe G-C-regions in nucleic acids. Binding studies with these labels and DNA suggest that these analogs bind to DNA and their DNA-binding modes are similar but not identical to the parent compound. These analogs inhibited DNA-dependent RNA polymerase and were more active than the actinomycin D against P-388 leukemia cells in vivo.

PHS-6040
(Rev. 10-76)

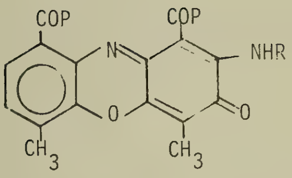
PROJECT DESCRIPTION

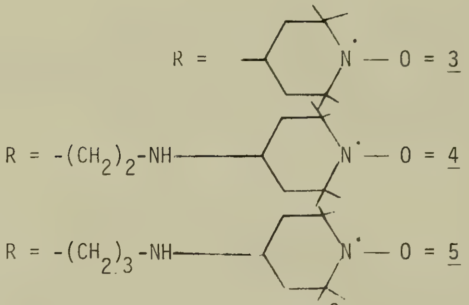
METHODS EMPLOYED: The purpose of the present work is to develop base specific probes for studying the binding of chemical carcinogens and mutagens to nucleic acids. Since, the binding of these agents to nucleic acids is base specific, it is of considerable interest to design and prepare base specific spin label probes to evaluate the mechanism of binding of carcinogens to nucleic acids and characterize the binding sites responsible for carcinogenesis.

Actinomycin-D (AMD) is one of the few antitumor agents possessing curative effects against Wilm's tumor and gestational choriocarcinoma. The mechanism of action of Actinomycin-D appears to result from intercalation of flat ring systems into G-C base pairs of double-standard DNA which leads to an inhibition of protein synthesis. The base specificity of Actinomycin-D in binding to DNA, has prompted us to design and prepare labels (3-5) to probe the binding of certain carcinogens to the G-C base pairs.

Where P = Thr-D-Val-Pro-Sar-Me-Val

When R = H = Actinomycin-D = 1





MAJOR FINDINGS AND PROPOSED COURSE: We have synthesized N^2-[4-(2,2,6,6-tetramethyl-1-piperidinyloxyl)] actinomcyin D and the related 1,2-diaminoethane and 1,3-diaminopropane derivatives (3-5) and have evaluated their biological properties. Binding studies with the spin labeled actinomycin D analogs and DNA were carried out by using circular dichroism, electron spin resonance and thermal denaturation. The electron spin resonance and circular dichroism studies with spin labeled AMD indicate that the phenoxazone rings of 3 and 4 are not intercalated into double stranded DNA, possibly due to steric effects; however, intercalation may be involved in the binding of 5. The peptide conformations in 3, 4 and 5 are similar but not identical to AMD. T_m measurements confirm the ESR and CD data and indicate a stronger binding of 5 to DNA as compared to 3 and 4. DNA-dependent RNA polymerase studies using either calf thymus DNA or *Micrococcus luteus* DNA as a template indicated similar activities for 3, 4 and 5 (4-7 μg/mL for IC_{50}) whereas AMD (IC_{50} = 0.2 μg/ml) was considerably more active, suggesting a stronger complex formation with double stranded DNA. Antitumor activity data against the P-388 tumor in mice indicate that 3, 4 and 5 are more active than AMD. At doses producing no toxicity 4 and 5 exhibited antitumor activity comparable to that of AMD while 3 was considerably more active.

The weak binding of 3-5 with double stranded DNA and their poor inhibitory activities against DNA dependent RNA polymerase are consistent with the theory that bulky substitutions in the 2 position greatly reduce intercalative binding. However, the high antitumor activities with little or not toxicity exhibited by 3, 4 and 5 are somewhat puzzling. The answers may then lie with the nature and conformations of the DNA-drug complexes, relative rates of dissociation and the specific interactions of the peptides with the DNA grooves. In addition, *in vivo* metabolism of these compounds to some active derivative may also be important. Bachur *et al*. have recently shown that AMD is metabolized to a free radical which augments oxygen consumption with the formation of superoxide. The biological activity of AMD may then be related to the superoxide formation. It is tempting to suggest that the increased antitumor effectiveness of derivatives 3-5 may also be due to the *in vivo* generation of superoxide.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since the binding of many chemical carcinogens to nucleic acids is thought to be base specific, it is hoped that by preparing and utilizing base specific spin labeled probes, we would be able to define and understand the interaction of carcinogens with nucleic acids at a molecular level. In addition, these probes will also aid in understanding the binding of histones and repressor proteins to nucleic acids.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50031-02 LEB

PERIOD COVERED
October 1, 1978 - September 30, 1979

TITLE OF PROJECT (80 characters or less)

Molecular Mechanisms of Mast Cell Degranulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Mary J. Ortner	Staff Fellow	LEB	NIEHS
OTHER:	Colin F. Chignell	Chief	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The membranes of both purified mast cells and mastocytoma cells have been studied using spin-labeled probes. The membrane fluidity of the neoplastic cells was significantly greater than that of the normal mast cells. The character of the spin probe was also found to be important in determining the accuracy of fluidity studies. Highly charged probes (such as fatty acids) may report a lower apparent fluidity due to the anchoring influence of their charged end group. Compound 48/80, a mast cell degranulating agent, had no effect on any of the lipid spin labels studied; however it was able to reverse the effect of magnetic interactions between closely adjacent spin labels. This suggests that 48/80 may increase the available membrane binding sites for the spin labels. Compound 48/80 also affected the state of MSL-ghost proteins by causing an increase in the population of highly immobilized label. The effect of trypsin was similar between MSL-ghosts and SL-48/80-labeled mast cells. Fluorescence microscopy has shown that mast cells and mastocytoma cells bind R-48/80 on the plasma membrane at low concentration and at high concentration, the binding also occurred at anionic sites in the cell interior.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Rat mast cells were isolated according to well established procedures and purified by centrifugal elutriation. The mastocytoma cells were an established cell line. The effect of 48/80 on the environment of 5-doxylstearic acid (5-DSA) and 5-doxylmethylstearate (5-DMS) was determined using the method in which the half width of the high field hyperfine extrema was measured at half its height. Rotational correlation times and order parameters were also calculated for the spin-labeled fatty acids. Fluorescence microscopy was carried out with a Leitz Dialux 20 microscope equipped with pleopak incident light fluorescence illuminator.

MAJOR FINDINGS AND PROPOSED COURSE: The rat peritoneal mast cell has been widely studied because of its role in the anaphylactic response and because it is a useful model system for examining the secretory process. We have studied the membranes of rat mast cells and peritoneal and pleural cells and also murine mastocytoma cells using the spin label method and fluorescence microscopy. We have also studied the effect of compound 48/80, a highly potent and efficacious stimulator of mast cell secretory action. For our electron spin resonance (ESR) studies, large numbers of purified mast cells were needed. We have therefore investigated the technique of centrifugal elutriation to provide rapid purification of large numbers of cells. Human erythrocyte ghost membranes covalently labeled with a maleimide spin label have also been used to study the interaction of 48/80 with the membrane proteins.

The effect of 48/80 concentration on cell binding was determined by fluorescence microscopy. Rhodamine labeled 48/80 (R-48/80) was studied at various concentrations using both mast cells and mastocytoma cells. Using this technique we were able to observe and photograph the binding of 48/80 to various cell types.

Rat peritoneal mast cells which constitute 6-10% of the peritoneal and pleural cell population can be successfully and rapidly purified using centrifugal elutriation. It was necessary to include serum albumin for the cells to retain their ability to secrete histamine. Elutriated mast cells released histamine in a manner similar to cells stored in the presence of albumin.

The ESR spectra of 5 DMS in both mast cells and mastocytoma cells suggested that the label was more highly immobilized in the mast cells than in the mastocytoma cells. In contrast, 5 DSA was very highly immobilized in both mast cells and mastocytoma cells and there was no apparent difference between the two cells types. The motion of the nitroxide group attached to stearic acid was very dependent on the position of the nitroxide with regard to the charged end of the molecule. There was a progressive increase in the motion of the nitroxide as it was located further from the ionized carboxyl group and, presumably further into the hydrophobic region of the cell. The signals from the spin labeled methyl stearates show a similar pattern; however, their motion was not as strongly influenced by the position of the nitroxide. In mastocytoma cells, all of the labels except the 5 DSA were less immobilized than in the mast cells. Also, in each case the nitroxide attached to the acid was more highly immobilized than its counterpart attached to the ester. However, this difference decreased as the nitroxide group was moved further from the charged end of the molecule.

The correlation times derived from spin labels in mast cell depleted leukocytes indicate that mast cell membranes are similar in fluidity to the other assorted white blood cells found in the rat peritoneal cavity.

Our data show that the apparent membrane fluidity increases as the reporter group is located further into the hydrocarbon region and further away from the charged end of the molecule. This finding is consistent with the current theories regarding membrane structure in that the most molecular ordering occurs at the surface and interface regions, presumably due to the presence of peripheral proteins and the glycerol backbone of phospholipids. In both mast cells and mastocytoma cells, the molecular ordering as reported by the 5 DSA was similar. However, the rotational correlation time (and presumably the membrane fluidity) increases very rapidly in the mastocytoma cells as reported by the signal from the 12th carbon, and there is little change from the 12th to the 16th carbon. In the mast cell, the increase in fluidity was more gradual and the abrupt change came between the 12th and the 16th carbon atoms. This effect was more pronounced with the acid derivatives, although the ester derivatives also showed a similar pattern. This finding indicates a fundamental difference between the mast cell and the mastocytoma cell membrane; however its significance if any, in explaining the neoplastic character of the mastocytoma cells, remains unclear.

The difference in motion seen between the acid and ester derivatives was greatest when the nitroxide was placed nearest the acid or ester group (5-DMS, 5 DSA), and diminished as the nitroxide was situated further away (16 DMS, 16 DSA). This was seen with both mast cells and mastocytoma cells and is probably due to the stronger "anchoring" effect of the acid moiety. The charge on the stearic acid therefore provides an additional constraint on the motion, and consequently, the apparent membrane fluidity is lower when the reporter group is situated close to its influence. If it can be assumed that the acid and the ester have the same orientation within the membrane, then the stearic acid probes were reporting artifically low fluidities for both the 5-nitroxide and the 12-nitroxide derivatives.

The current study shows that the mastocytoma cells are probably more fluid in all regions of the membrane. The 5 DSA was the only probe to report a similar fluidity for both cell types, however this was probably due to the strong influence of the charged group anchoring the molecule rather than the actual condition of the membrane. The 5 DMS probe suggests that mast cell membranes are indeed more rigid at the interface and this difference persists into the hydrophobic region of the membrane. This study shows that the use of 5-doxyl stearic acid to compare different membrane types can lead to erroneous results, and that several different probes should also be included in a complete study of membrane fluidity.

There was no effect of 48/80 on stearic acid and ester spin labels incorporated into either mast cells or mastocytoma cells; and the apparent molecular motion of the spin labels was not changed even at 48/80 concentrations far exceeding that which was maximally active. The effect of spin label concentration in the membrane has been studied. When the cells were incubated with spin label concentations higher than 2.5 x 10^{-5} M, the extrema of the labeled cells were broadened, presumably due to magnetic interactions between closely adjacent nitroxide molecules. The data showed that the effect of 48/80 on the spectra was only apparent in cases where there were magnetic interactions present. Compound 48/80 reduced the magnetic interactions and the spectra resumed a normal shape. There was no effect of 48/80 on cells in which the membrane concentration of label was magnetically dilute.

Although compound 48/80 precipitates a major molecular rearrangement in mast cell membranes, it was not possible to demonstrate any effect of this agent on bound stearic acid and ester probes which were magnetically dilute. Therefore, the absence of any effect by 48/80 on the labels suggests strongly that its action does not result in a permanent major fluidity change among the membrane lipids or at the interface.

The effect of 48/80 on high concentrations of the stearate spin-label in the mast cell membrane indicates that 48/80 increased the distance between interacting spin-labeled molecules, thereby reducing intermolecular magnetic interactions. One possible explanation for this observation is that compound 48/80 increases the number of binding sites for the spin labels. We have previously suggested that 48/80 may act by causing a partial denaturation of the membrane proteins. If this mechanism is correct, the denatured membrane proteins may provide the additional binding sites which are generated by 48/80.

Spin-Labeled Ghosts: We have spin-labeled ghost membranes using a maleimide derivative (MSL) which covalently binds to membrane proteins via their sulfhydryl groups. We have found that 48/80 causes an increase in the population of more highly immobilized spin labels. These results are direct evidence of a membrane protein interaction by 48/80. We have also found that treatment of MSL-ghosts with trypsin causes rapid destruction of the highly immobilized sites. Similar treatment of purified mast cells labeled with spin-labeled 48/80 yields the same result. These data indicate that 48/80 binds to a general site on cell membranes which may be similar to that of the MSL and is probably a protein.

Fluorescence Microscopy: Compound 48/80 was covalently labeled with the fluorescent moiety, rhodamine. At low concentrations rhodamine 48/80 (R-48/80) binds to the plasma membrane of both mast cells and mastocytoma cells. In some cases we have observed "patching" of the fluorescence intensity which would indicate aggregation of the receptor sites. At high concentration, R-48/80 bound to anionic intracellular structures. The R-48/80 apparently increases the membrane permeability, allowing access to the cell's interior. In mast cells, the R-48/80 bend to the basophilic granules, whereas in mastocytoma cells it binds to the nucleus.

We have made a detailed study of mast cell and mastocytoma cell membranes in order to understand better the underlying mechanisms behind non-cytotoxic histamine secretion. We have found significant differences between their membrane fluidities. We have also shown that mast cell membranes are similar in fluidity to that of the other normal cells found in the peritoneal and pleural cavities and that this fluidity is unaffected by 48/80. This indicates that the ability to secrete histamine is unrelated to membrane fluidity. In addition we have shown through fluorescence microscopy that 48/80 binds to the outer membrane at low concentrations and to inner constitutents at higher levels. We are currently expanding our studies to include the effect of 48/80 on the proteins of ghost membranes as studied by circular dichroism. We also plan to study individual proteins in their solubilized form using the same procedure.

The studies should contribute to our knowledge of the apparent protein denaturation caused by 48/80. We are also continuing our studies using the spin labeled maleimide ghosts. By studying the effects of 48/80 on membrane proteins directly we hope to characterize more precisely its molecular interaction. We intend to expand our fluorescence microscopy studies to determine the effect of heat treatment and trypsin on the binding of 48/80. Furthermore we will also study the effects of pharmacological treatments such as colchicine and cytochalasin B and also drugs which are known to compete for 48/80 binding sites such as polymyxin B. We are therefore continuing the work of our previous year in order to identify the binding site and molecular mechanism of 48/80.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Immunological stimulation of histamine secretion via IgE antibody-antigen complex formation mediates the symptoms of asthma, hay fever and anaphylactic shock. In addition to this reaginic response, histamine secretion can also be initiated by several drugs and chemicals. Morphine curare, chlorpromazine, concanavalin A, dextron, calcium conaphores and 48/80 are among the many agents in this catagory. Histamine secretion by non-immunological means therefore presents a serious health hazard to those who are exposed to such drugs for therapeutic reasons. Mast cells are also implicated in the inflammatory reactions associated with photoallergic and phototoxic dermatitis. In addition they may also exacerbate the conjunctivities and pulmonary distress caused by certain xenobiotics in the atmosphere. With the aid of biophysical techniques and compound 48/80, we are studying the molecular mechanism of histamine secretion. These studies may lead to the development of a safe method of control over both immunologically and environmentally related adverse conditions due to histamine secretion by mast cells.

PUBLICATIONS

Ortner, M.J., Sik, R.H., Chignell, C.F. and Sokoloski, E.A.: A nuclear magnetic resonance study of compound 48/80. Mol. Pharmacol. 15: 179-188, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50032-02 LEB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Binding of Copper to Human Serum Albumin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Mohanakrishnan	Visiting Fellow	LEB	NIEHS
Others:	Colin F. Chignell	Chief	LEB	NIEHS

COOPERATING UNITS (if any)

NONE

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The interaction of copper with human serum albumin is being examined with the aid of a number of spectroscopic techniques. In addition to albumin itself, binding studies are also being made with the N-terminal tripeptide of albumin and with larger peptide fragments isolated from enzymatic digestion of albumin. In these studies the amino acids that function as chelation sites for copper will be identified. This work will provide a molecular basis for the interaction between copper and human serum albumin in both normal and diseased states (Wilson's disease, rheumatoid arthritis and Bechterew's disease).

PROJECT DESCRIPTION

METHODS EMPLOYED: The binding of metal ions to human serum albumin (HSA) was carried out using fluorescence spectroscopy. A copper ion (Cu^{2+}) specific electrode was used to study the binding of copper to albumin from different animal sources. Kinetic studies of the binding of copper to HSA was performed using stopped flow fluorescence spectrometry. HSA was labeled with fluorescent labels. Attempts were made to synthesize peptides with the same amino acid sequence as the N-terminus of HSA.

MAJOR FINDINGS AND PROPOSED COURSE: The metal ions used in binding studies with HSA spectrofluorometrically were Cu^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+} and Zn^{2+}. Only Cu^{2+} was found to quench the tryptophan fluorescence of HSA. Stern-Vollmer plots indicated that the mechanism of fluorescence quenching is predominantly energy transfer. Comparison of the results from HSA and canine serum albumin indicated that the copper binding sites besides the N-terminal copper-binding site in HSA also contribute to fluorescence quenching.

The binding of copper to human, rabbit, canine and porcine albumins was measured using a copper ion specific electrode. It was found that there are two very high affinity copper binding sites for HSA characterized by association constants of the order $2 \times 10^6 M^{-1}$. There are about seven relatively less affinity binding sites, the association constant in this case being one order less.

Stopped flow studies of the kinetics of copper binding to HSA were carried out under pseudo first order conditions and equimolar amounts. The results were analyzed using a one-term expression. It was found that the second order rate constant is nearly $5 \times 10^5 M^{-1} S^{-1}$. Since the studies with Cu^{2+} ion specific electrode indicated that there are multiple binding sites, the results will have to be reexamined in terms of a two term expression.

Attempts to synthesize the tripeptide, Asp-Ala-His-$NHCH_3$, were unsuccessful because N- and β-carboxyl protected aspartic acid used to couple to the dipeptide Ala-His-NHMe by an active ester coupling procedure underwent cyclization reactions, as reported by Bodlansky. Hence the synthesis of the tripeptide will be carried out with dicyclohexylcarbodiimide as the coupling agent.

HSA was covalently labeled with dansyl and pyrene derivatives. The label emission spectra were observed by activating the intrinsic fluorophore, tryptophan. The labeled HSA will be used to study the binding of metal ions and other ligands.

The binding of Cu^{2+} to HSA was also measured by NMR using ^{79}Br as the probing nucleus. Preliminary studies showed a broadening of the bromide ion signal from 0.2 M KBr containing HSA in the presence of copper. Further studies are in progress.

Since there are several copper binding sites for HSA, it is necessary to know the types of amino acid groups at these sites in order to understand the mechanism of binding in detail. It is therefore proposed to undertake further NMR studies employing high frequency NMR spectrometers. NMR studies of the fragment peptides of HSA will also be carried out. Binding of drugs and other small molecules to HSA will also be made using NMR and fluorescence and spin label techniques with a view to obtaining some idea of the three-dimensional structure of the protein and its combining sites.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The waste effluents from several energy-related processes including oil shale production and coal gasification contain high concentrations of heavy metal ions including Cu^{2+}. The binding of heavy metals by human serum albumin has important toxicological implications since this protein can act as both a carrier and a "sink" for these ligands. Thus it is of paramount importance to understand the factors which govern the interaction between metal ions and serum albumin.

PUBLICATIONS

Boobis, S.W. and Chignell, C.F.: Effect of protein concentration on the binding of drugs to human serum albumin I sulfadiazine, salicylate and phenylbutazone. Biochem. Pharmacol. 28; 751-756, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50033-02 LEB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Do Nitroaromatic Free Radicals Covalently Bind to Macromolecules?

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ronald P. Mason	Research Chemist	LEB NIEHS
Others:	B. Kalyanaraman	Visiting Fellow	LEB NIEHS
	Carl Polnaszek	Research Chemist	Mpls. VA Hosp.
	J.L. Holtzman	Pharmacologist	U. of Minn.

COOPERATING UNITS (if any)

Department of Pharmacology, University of Minnesota

LAB/BRANCH

Laboratory of Environmental Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, North Carolina

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have proposed that nitroaromatic anion free radicals ($\mathrm{R\dot{N}O}_2^-$) covalently bind to glutathione, protein or to other tissue macromolecules,

$$\mathrm{R\dot{N}O}_2^- + \mathrm{P} \longrightarrow \mathrm{RNO}_2 - \mathrm{P}$$

where P represents a macromolecule or glutathione. In most studies on the covalent binding of reductive intermediates of nitro compounds to protein it is usually assumed that the hydroxylamine metabolite is the species that covalently binds. Yet there is only very circumstantial evidence to support this assumption. Since as much as 14% of a reductive intermediate of some nitrofurans becomes covalently bound to protein, it should be possible to observe spectroscopically the covalent binding of the anion radicals, if this intermediate is indeed responsible for binding. Because reduced glutathione depletion is thought to be important in some drug toxicities, it is important to determine if the anion radical cal directly bind to glutathione. In addition, L-cysteine or GSH decreased the covalent binding of one nitrofuran carcinogen by 99.9% without affecting the microsomal nitroreductase activity. Just recently the binding of a reductive metabolite(s) of two nitrofurans to DNA and RNA has been reported.

PROJECT DESCRIPTION

METHODS EMPLOYED: Electron spin resonance (ESR) has been utilized to detect steady-state levels of nitrocarcinogen free radicals.

MAJOR FINDINGS AND PROPOSED COURSE: We wish to examine the effect of added glutathione, protein, DNA and RNA on nitrofuran anion radical kinetics. The steady-state concentration of anion radical as determined by an ESR spectrometer, will be altered by protein concentration if the radical binds to protein. Covalent binding of RNO_2^- to glutathione, protein, or other macromolecules, P, will compete with the disproportionation of the radical as a decay mechanism according to the following rate expression,

$$\frac{d[R\dot{N}O_2^-]}{dt} = k_1\,[E]\,[RNO_2] - 2k_d\,[R\dot{N}O_2^-]^2 - k_c\,[R\dot{N}O_2^-]\,[P],$$

where k_c is the rate of covalent binding by the anion radical to protein, and $k_1\,[E]\,[RNO_2]$ is the rate of enzymatic radical formation.

$$R\dot{N}O_2^- + P \xrightarrow{k_c} RNO_2 - P$$

The steady state condition yields

$$[R\dot{N}O_2^-]_{ss} = \frac{-k_c\,[P] + \sqrt{k_c^2\,[P]^2 + 8k_dk_1\,[E]\,[RNO_2]}}{4k_d}$$

Any reversible association between P and E or RNO_2 will be reflected in k_1. If k_1 is unchanged by increasing P, but the steady state radical concentration is reduced by increasing P, then RNO_2^- is probably covalently binding to P.

Work in Dr. Holtzman's laboratory indicates that 100 mM GSH does not affect the steady-state concentration of a nitrofuran carcinogen free radical in microsomal incubations. Published work indicates that the GSH does not affect microsomal nitroreductase activity, but can decrease protein binding by 99.9% at 0.4 mM. This result indicates that RNO_2^- is not the reductive metabolite(s) that covalently binds to protein. Neither DNA nor bovine serum albumin decrease the free radicals concentration. RNO_2^- does not appear to react with GSH, protein, or DNA. On the other hand, RNA (8 mg/ml) decreased the radical concentration by 50% in a concentration dependent fashion. The rate of reduction of the nitrofuran carcinogen by xanthine oxidase was increased from 7.98 ± 0.13 nmole/min with RNA. These data suggest that the anion radical reacts with RNA.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The nitro carcinogens, five of which are available from NCI's chemical repository, are thought to require reductive activation before they can initiate tumor formation. The binding of a reductive intermediate to tissue macromolecules is thought particularly important in this regard. The investigation of the possible reactions between RNO_2^- and GSH or macromolecules contributes to our understanding of the mechanism of nitro activation.

Our results support the conclusions of other workers, who have proposed that the reactive intermediate is the hydroxylamine metabolite. Although the chemical structure of the covalently binding metabolite is still unknown, our work has eliminated the nitro radical anion as a possible candidate.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50034-02 LEB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Mechanism of the Oxygen-Insensitive Activation of 4-Nitroquinoline-1 oxide

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ronald P. Mason	Research Chemist	LEB	NIEHS
Others:	B. Kalyanaraman	Visiting Fellow	LEB	NIEHS

COOPERATING UNITS (if any)
NONE

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

With one exception all mammalian nitroreductases are markedly inhibited by oxygen. Previous investigations have found that the oxygen-insensitive nitroreductase of rat liver supernatant is DT diaphorase. The reduction of 4-nitroquinoline-1-oxide (4-NQO) by supernatant was inhibited by oxygen only 10%, whereas dicoumarol inhibited 94%. The microsomal reduction of 4-NQO is oxygen sensitive as is characteristic of the usual nitroreductases. Several lines of evidence suggest that the oxygen inhibition of nitroreductases is the result of the air oxidation of the first reduction intermediate, the nitroaromatic anion free radical (RNO_2^-).

$$RNO_2^- + O_2 \longrightarrow RNO_2 + O_2^-$$

In view of the known rapid rate of the air oxidation of nitro anion free radicals ($k > 2.5 \times 10^5\ M^{-1}\ sec^{-1}$), this oxygen-insensitive nitroreductase must not form the RNO_2^- or, at the very least, the radical must remain bound to the DT diaphorase and not be accessible to O_2. Presumably in this case, the first reduction intermediate formed is the corresponding nitroso compound, the two-electron reduction product.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Electron spin resonance was used to search for the anion free radical metabolite of 4-nitroquinoline-1-oxide (4-NQO). Oxygen uptake and the rate of NADPH oxidation where determined using standard techniques. The adrenochrome assay used for superoxide is a modification of the method of Misra and Fridovich.

MAJOR FINDINGS AND PROPOSED COURSE: If the mechanism of microsomal nitroreduction of 4-NQO is like that of other oxygen-sensitive nitroreductases, then anaerobic incubations of microsomes, 4-NQO and NADPH should form the anion free radical, which should be detectable by electron spin resonance. Under aerobic conditions the free radical should not be detectable, but 4-NQO should stimulate O_2 uptake, superoxide formation, and NADPH oxidation by the microsomal incubation via the cyclic formation of the nitro anion free radical.

In contrast, we expect that incubations containing an equal amount of supernatant nitroreductase activity will not form the 4-NQO anion free radical, as determined by electron spin resonance. Again, in contrast to the microsomal incubations, the supernatant incubation should not consume more oxygen or oxidize more NADPH in the presence of 4-NQO.

Lastly, supernatant incubations should not form more superoxide in the presence of 4-NQO, whereas microsomal incubations are expected to generate more superoxide. Since the nitro compound-catalyzed oxygen consumption, superoxide formation and NADPH oxidation are thought to be due to the rapid air oxidation of $RNO_2^{}$, these results would support the electron spin resonance results concerning the formation of the 4-NQO anion radical in the microsomal and supernatant incubations.

The 4-NQO radical anion was very difficult to detect even in microsomal incubations. The spectrum was very broad, and was typical of an immobilized free radical. This factor contributed to the difficulty in observing this species. The 4-NQO radical anion must be bound to the microsomes, and not free rotating in the buffer as are other nitro anion free radicals which we have investigated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Either the nitroso or hydroxylamine metabolites of 4-NQO are thought to be the proximate carcinogen of 4-NQO. The rapid cellular reduction of 4-NQO in air is unique among nitro compounds and is thought responsible for its greater toxicity and mutagenicity and for its potent carcinogenicity relative to that of other nitro compounds.

PUBLICATION

Peterson, F.J., Mason, R.P., Housepian, J. and Holtzman, J.L.: Oxygen-sensitive and insensitive Nitroreduction by E coli and Rat Hepatic Microsomes. J. Biol. Chem. (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50035-02 LEB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Interpretation of the ESR Spectra of Spin-labeled Membranes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ronald P. Mason	Research Chemist	LEB NIEHS

COOPERATING UNITS (if any)

NONE

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

McConnell, et al. have developed an S parameter method of spectral interpretation which is almost universally used in the analysis of spin-labeled membrane spectra. Hubbell and McConnell have emphasized that for their S formalism to be valid, one must have Trace T = Trace T', where T is the nitrogen hyperfine tensor in the principal axis system and T' has the components $T_{\parallel}$ and $T_{\perp}$. This requirement follows directly from the rotational invariance of the trace of a tensor. In the work of Hubbell, et al variations in a_N, the isotropic nitrogen hyperfine splitting, have been interpreted as changes in the polarity of the local environment of the nitroxide group, and a correction has been used to adjust the measured S for the polar-hydrophobic effect. This polarity profile within the hydrocarbon region of the bilayer has been attributed to electrostatic interactions between the nitroxide dipole and the dipolar regions of the bilayer. Although the polarity profile does probably exist in many cases, a breakdown of assumptions made in the derivation of the S parameter formalism could cause an artifactual polarity change which is actually a violation of the necessary condition that Trace T = Trace T'.

PROJECT DESCRIPTION

METHODS EMPLOYED: All ESR spectra of microsomal membranes were recorded with a Varian E-109 spectrometer equipped with a Varian TM_{110} cavity. Temperatures other than 22°C were achieved with a Varian temperature controller using the variable temperature flat cell and dewar designed for the TM_{110} cavity. Both the microwave power and the modulation amplitude were set at one-tenth of the values necessary to maximize the intensity of the center line signal.

MAJOR FINDINGS AND PROPOSED COURSE: The isotropic nitrogen hyperfine splitting, a_N, has been found to be independent of temperature in a variety of organic solvents. Even rigid limit spectra taken at liquid nitrogen temperature have the same a_N as spectra at room temperature or above. The parameter a_N for spin-labeled membranes has been reported to be a function of temperature. The result has been thought to represent a real change in the polarity profile of the membrane. We have measured a_N for spin-labeled egg lecithin as a function of temperature in the vicinity of room temperature and at liquid nitrogen temperatures where a_N can be determined independently of motional effects. We expected that at liquid nitrogen temperatures a_N would be temperature independent and different from the apparent value determined by the extrapolation of a_N values near room temperature, which will be determined using the Hubbell-McConnell Formalism.

Unfortunately the ESR spectra of spin-labeled membranes were too poorly resolved to determine a_N at low temperatures. Although the expected trends in S and a_N were observed above 0°C, we could not interpret this results without the low temperature data. This project has been discontinued.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many workers in the biological sciences are using spin labeling to investigate phenomena which are thought or have been shown to be related to membrane fluidity, such as cancer, muscular dystrophy, complement-induced membrane damage and the mechanism of action of lytic compounds. Changes in the apparent a_N are often described in these studies. In many of these studies an increase in a_N is accompanied by an increase in S. Although rigorous simulations of spectra have shown that changes in the rotational rate of the nitroxide about its symmetry axis of rotation can cause simultaneous changes in a_N and S of the kind reported in the literature, experimental proof is needed of artifactual changes in a_N.

PUBLICATIONS

Mason, R.P. and Polnaszek, C.F.: Spin-Label and Deuterium Order Parameter Discrepancies in Bilayers: One Possible Explanation. Biochemistry 17: 1758, 1978.

Giavedoni, E.B., Mason, R.P. and Dalmasso, A.P.: Complement-Induced Modifications in Membrane Fluidity: Studies with Resealed and Glutaraldehyde-Treated Erythrocyte Membrane Ghosts. Journal of Immunology. 120: 2003-2007, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50036-02 LEB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

A Search for Free Radical Metabolites of Polycyclic Hydrocarbons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Kalyanaraman	Visiting Fellow	LEB	NIEHS
OTHERS:	Ronald P. Mason	Research Chemist	LEB	NIEHS

COOPERATING UNITS (if any)

NONE

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina

TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We propose to use electron spin resonance to search for free radical metabolites of polycyclic hydrocarbons and dibenzodioxins. Free radicals of electron donor molecules are formed in the peroxidase reaction according to the following mechanism.

$$\text{Peroxidase} + H_2O_2 \longrightarrow \text{Compound I} + H_2O$$

$$\text{Compound I} + R \longrightarrow \text{Compound II} + R^+$$

$$\text{Compound II} + R \longrightarrow \text{Peroxidase} + R^+$$

Many compounds are known to undergo such a one-electron oxidation in the presence of a variety of peroxidases and hydrogen peroxide. Electron spin resonance spectra have been obtained of the free radical metabolites of hydroquinone and N-hydroxy-2-acetylaminofluorene and many biological compounds. This class of enzymes appears to have little substrate specificity, any electron donor with a sufficiently low oxidation potential should be metabolized to a free radical. In addition to lactoperoxidase and myeloperoxidase, hemotin, methemoglobin and cytochrome P-420 all have peroxidase activity.

PROJECT DESCRIPTION

METHODS EMPLOYED: Electron spin resonance (ESR) has been utilized to detect free radical intermediates. Difference absorption spectra have been used to follow the formation and decay of compound I and II.

MAJOR FINDINGS AND PROPOSED COURSE: Ten μl/ml of 30% H_2O_2 was added to an incubation mixture containing horseradish and the tetrasulfonic acid derivative of pyrene in acetate buffer (0.2 M pH 4.6). The incubate was then aspirated through a stainless steel needle into an aqueous flat cell mounted in the microwave cavity of the ESR spectrometer. The ESR spectrum at room temperature was immediately determined. The exploratory investigations on pyrene and benz[a]pyrene were unsuccessful in that an ESR spectrum was not observed. Furthermore no color change was observed as would be expected if benz[a]pyrene was being oxidized to a quinone. Quinone products would be expected in this free radical pathway. A review of the literature did not reveal any reports of polycyclic hydrocarbon oxidation by peroxidases, so this project was concluded.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The metabolic activation of aromatic hydrocarbons by one-electron oxidation has been proposed to be of importance in the mechanism of tumor initiation, but no one has presented evidence for the enzymatic formation of the proposed cation free radicals. The formation of the analogous cation free radical of 2,3,7,8-tetrachlorodibenzo-p-dioxin has also been postulated as the oxidative metabolite which binds to rat liver microsomes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50037-01 LEB

PERIOD COVERED
June 1979 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effect of 2450 MHz Microwaves on Plasma and tissue Enzymes of Guinea Pigs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael J. Galvin	Senior Staff Fellow	LEB	NIEHS
	Donald I. McRee	Research Physicist	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Non-Ionizing Radiation Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.2	.1	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project examines the interaction of 2450 MHz microwaves with guinea pigs exposed in vivo to whole body radiation. Animals were exposed for four days, six hours per day to either 5, or 30 mW/cm^2 of microwave radiation. On day five the animals were sacrificed and certain tissue and plasma enzymes were monitored. In these experiments the temperature and humidity of the environmental chambers were carefully monitored in order to eliminate any effects not intrinsically caused by microwave radiation. No data is currently available on the effects of microwave exposure. However during this time, the methodology for the exposure of guinea pigs to microwave radiation has been developed.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPITION

METHODS EMPLOYED: Male guinea pigs weighing between 400 and 500 grams are exposed to microwave radiation at carefully controlled temperatures and exposure levels. The animals are exposed for four days six hours per day at at 5, or 30 mW/cm^2. On the fifth day the animals are sacrificed and the tissue and plasma levels of the following enzymes are measured: a. Lactic Acid Dehydrogenase, b. Cathepsin D, c. Beta glucuronidase and d. Glutamic-oxaloacetic transaminase.

The results are expressed as enzyme units per unit of protein. In addition the total and differential white, blood cell counts and blood hematocrits are determined.

MAJOR FINDINGS AND PROPOSED COURSE: During the past year this work has focused on designing an apparatus for exposing guinea pigs to 2450 MHz cw microwaves. This work has resulted in the development of a microwave exposure system for guinea pigs. However no data is yet available on the specific parameters being measured in this test system.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Before an accurate evaluation of the biological effects of 2450 MHz microwaves can be made, it is necessary to control carefully the environment of the organism being exposed. We have developed a system for carefully monitoring and reproducing identical environmental conditions for both exposed and control guinea pigs. This system will therefore allow us to make more accurate assessments of potential effects of microwaves in biological systems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50038-01 LEB

PERIOD COVERED

January 1, 1979 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of 2450 MHz Microwave Radiation on Cultured Heart Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael J. Galvin	Senior Staff Fellow	LEB	NIEHS
	Donald I. McRee	Research Physicist	LEB	NIEHS
Other:	Melvyn Lieberman	Consultant	LEB	Duke University

COOPERATING UNITS (if any)

Physiology Department, Duke University

LAB/BRANCH

Laboratory of Environmental Biophysics

SECTION

Non-Ionizing Radiation Workgroup

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to determine how 2450 MHz microwave radiation interacts with biological material at the cellular level, to observe any effects of this interaction, and to relate the amount of microwave energy absorbed to the effect. The biological system employed in the study is cultured heart cells obtained from 8 day old quail and maintained in culture for two days. In these experiments, the temperature, humidity, and other factors have been carefully monitored in order to eliminate any effects not directly caused by microwave radiation. The design of this exposure system will be completed soon. Experiments are planned for this new test system as soon as the experimental design is completed.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Cultured quail heart cells are exposed to microwave radiation at carefully controlled temperatures and exposure levels. Cultures are exposed to 5 and 30 mW/cm^2 levels of microwaves for 1 or 3 hours. Both light and electron microscopy techniques are employed to determine microwave effects. In addition changes in certain biochemical parameters are also monitored.

MAJOR FINDINGS AND PROPOSED COURSE: This project requires the design of a microwave exposure system for cells maintained in a sterile, temperature and humidity controlled environment. Several methodologies are being examined for exposing cultured heart cells to microwave radiation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Before an accurate evaluation of the biological effects of 2450 MHz microwaves can be made, it is necessary to control the temperature of the specimen carefully and be able to reproduce the exposure conditions. We are developing the capability to do this at NIEHS and, this may provide a system for differentiating specific microwave effects from thermal responses.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50039-01 LEB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
The Effect of 2450 MHz Microwave Radiation on Mast Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Mary J. Ortner	Staff Fellow	LEB	NIEHS
	Michael J. Galvin	Senior Staff Fellow	LEB	NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics/Non-Ionizing Radiation Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1		

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effect of microwave radiation was studied on active secretory cells. Rat peritoneal mast cells were exposed to 2450 MHz microwave radiation at specific absorption rates (SAR) of 8.5 and 42.5 mW/ml for periods of up to 3 hrs. Cells were maintained throughout exposure at 37°C. There was no effect on all viability or spontaneous histamine release. Mast cells exposed to compound 48/80 after prior irradiation or during simultaneous irradiation secreted histamine in a manner similar to unexposed cells.

PROJECT DESCRIPTION

METHODS EMPLOYED: Rat peritoneal mast cells were obtained by peritoneal cavity lavage. The cells were irradiated in a waveguide exposure system using a frequency fo 2450 MHz. Dosimetry in the cell chamber was determined directly from the time-temperature profiles to be 8.5 and 42.5 mW/ml respectively. The temperature during exposure was maintained at 37°C using a circulating water bath. Control cells were incubated 9.5 cm away from the microwave source and were not exposed to the radiation. After irradiation, samples of cells were removed and added to either Lockes solution or compound 48/80. Histamine release was measured using the fluorometric method of Shore.

MAJOR FINDINGS AND PROPOSED COURSE: Mast cells were irradiated for 15, 30, 45 and 60 minutes at SAR of 8.5 mW/ml and exposed to compound 48/80 (2 µg/ml). Histamine release was similar in both control and irradiated cells at all time periods. The irradiated cells did not differ in spontaneous histamine release or cell viability as measured by trypan blue exclusion.

Mast cells were irradiated at a SAR of 42.5 mW/ml for periods of up to 3 hrs. There was no evidence that the increased SAR or incubation time had any effect on the stability of the cells or on the release of histamine. Compound 48/80 was also added to the mast cells during 42.5 mW/ml irradiation. The effect of simultaneous exposure was similar to prior exposure in that the cells responded normally to the histamine liberator. Dose/response curves showed that submaximal concentrations of 48/80 were as effective in irradiated cells as in the controls.

Mast cells which had been heated to 45°C for 10 min became totally unresponsive to 48/80. Cells from the same sample were exposed to microwave radiation at 42.5 mW/ml for 3 hrs. Unlike the heat treated cells, the irradiated cells were undiminished in their response to 48/80. This indicates that the microwaves did not produce damaging effects due to localized thermal gradiants across the membrane.

The present study will be expanded to determine the effect of microwaves on calcium uptake. This will be measured by studying histamine secretion induced by the ionophore A23187, which stimulates secretion by promoting calcium influx into the mast cells. Studies of calcium binding by A23187 within the cell membrane will also be done by measuring the fluorescence polarization of the ionophore.

The effect of microwaves on the membranes of erythrocyte ghosts will also be studied using circular dichroism (CD), which measures optical activity in a qualitative and quantitative manner. This technique has been widely used to determine the effect of drugs and chemical treatments (ie. detergent-induced protein denaturation) on membrane proteins. Since the CD spectrum of a protein is sensitive to changes in tertiary structure, this technique may be useful to measure the effects of microwaves on the cell membrane.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A thorough study of the effects of microwave radiation on all biological levels must be completed before microwave radiation exposure can be considered safe. Waveguide systems are being developed to study the effects of non-ionizing radiation on all membranes. The complicated series of events resulting in mast cell membrane fusion and histamine secretion are unimpaired by the dosage and frequency of microwave radiation used in these experiments. However, the sensitvity of this reaction to heat makes mast cells a potential biological dosimeter, providing evidence against the presence of damaging interface heating in the mast cell membrane after microwave exposure. The use of these cells should make it easier to identify the effects of microwave radiation which are unrelated to thermal damage.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50040-01 LEB

PERIOD COVERED
June 1, 1979 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Influence of 2450 MHz Microwave Radiation on Cats subjected to Myocardial Ischemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael J. Galvin	Senior Staff Fellow	LEB	NIEHS
	Donald I. McRee	Research Physicist	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Non-Ionizing Radiation Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: .3	PROFESSIONAL: .2	OTHER: .1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project examines the effects of 2450 MHz microwave radiation on the course of myocardial ischemia in cats. Certain biochemical and physiological parameters which are influenced by myocardial ischemia are followed in animals subsequent to myocardial ischemia. In conjunction with this, methods have been developed for determining the dose of microwave radiation absorbed by the myocardial tissue. The parameters measured include plasma and tissue creatinine phosphokinase levels, electrocardiogram changes, measurement of infarct size, and cardiac output and blood pressure subsequent to myocardial ischemia. In these experiments the temperature of the tissue has been carefully monitored in order to determine the thermal contribution of microwaves to the ischemic injury. No results are yet available.

PROJECT DESCRIPTION

METHODS EMPLOYED: Cats are subjected to myocardial ischemia by occlusion of the left anterior descending coronary artery. In some animals the ischemic myocardium is exposed to 2450 MHz microwave radiation at carefully monitored dosages of 5 and 30 mW/gm tissue and certain physiologic and biochemical indices monitored for five hours post occlusion. Appropriate control and experimental cats are also included in this study. Serial blood samples are taken every hour during the course of the experiment and at termination liver and myocardium tissue is biopsied. Tissues are examined for alterations in cellular integrity utilizing both histologic and biochemical techniques. Plasma creatine phosphokinase activity and the S-T segment of the electrocardiogram and arterial blood pressure are used as indicators of the severity of the infarct.

MAJOR FINDINGS AND PROPOSED COURSE: During the past few months a model has been developed to examine the interactions of 2450 MHz CW radiation on tissues which are ischemic. The model in this study is designed to examine the possible interactions between various levels of microwave exposure and ischemic mycardial cells. No results are available.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The potential health effects of microwave radiation in the environment is of interest to NIEHS. The effects of 2450 MHz microwaves may not be evident in normal tissue, but in ischemic tissue there may be influences from non-ionizing radiation. The use of cells which have been compromised may be a useful system for evaluation of possible microwave effects. This research on the effects of microwaves on ischemic injury is directed toward the mission of NIEHS to determine the health effects of physical factors in the environment.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50041-01 LEB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Permeability of the Cochlear Partition Assessed by Electrochemical Potential change during Anoxia.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Teruzo Konishi	Medical Officer	LEB	NIEHS
	Alec N. Salt	Visiting Fellow	LEB	NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.6	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Ionic permeability changes in the endolymph-perilymph barrier are significant factors in the physiological mechanisms underlying the noise-induced hearing loss. The purpose of this study is to estimate membrane permeability in normal and pathological cochlea by measuring alterations of the electrochemical potentials during anoxia.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Guinea pigs anesthetized with pentobarbital sodium were used. The endocochlear potential (EP) and K^+ concentrations of the endolymph and perilymph were simultaneously measured in the basal turn of the cochlea with a pair of double barreled K^+ selective microelectrodes. The sound induced responses were also recorded with differential electrodes placed in the basal turn. If the volume (V) of the endolymph is assumed to be constant during anoxia, the efflux (J) of K^+ from the endolymph can be expressed by

$$J=V\frac{d[K_{endo}]}{dt}$$

The K^+ conductance of the endolymph-perilymph barrier (G_K) is therefore the ratio of K^+ current to the electrochemical potential difference,

$$G_K=\frac{I_K}{\Delta\tilde{\mu}_K/F}$$

$$=\frac{\frac{d[K_{endo}]}{dt}\quad VF}{E_m+RT\ln\frac{[K_{endo}]}{[K_{peri}]}}$$

Three groups of guinea pigs were used; one group was treated with ototoxic antibiotics, either by local injection of dihydrostreptomycin (0.1g) into the auditory bulla or by daily intramuscular injection of kanamycin (400 mg/kg). The second group was exposed daily to broad band noise at 115 dBA for periods ranging from 3 to 17 days. A third group served as controls.

MAJOR FINDINGS AND PROPOSED COURSE:

1. Normal guinea pigs

The average EP fell from 85.0 mV to a minimum of -35.0mV at 3 min after onset of anoxia. Thereafter the magnitude of EP slowly decreased during one hour observation. The rate of K^+ concentration change in the endolymph was fast during 3 to 8 min period after onset of anoxia, ranging from 1.04 mM/min to 0.80 mM/min, and gradually decreased as the time elapsed. The average K^+ concentration in the perilymph was 3.4 mM and 30 min after anoxia it ranged from 11 to 18 mM. The K^+ conductance of the cochlear partition ranged from 11.5 to 15.8 5 min after anoxia and it gradually decreased as the anoxia period was prolonged.

2. Antibiotic treated animals.

The EP remained normal in guinea pigs treated with ototoxic antibiotics locally or systemically, wereas the sound induced responses were almost totally abolished. Changes in the electrochemical gradient for K^+ during anoxia were far less than those observed in normal animals. The rate of $[K_{endo}]$ decrease was also small. Thus the calculated K^+ conductance of the cochlear partition at 10 min after anoxia ranged from 1.03 to 4.80.

3. Noise exposed guinea pigs.

In 22 guinea pigs exposed to broad band noise at 115 dBA for periods ranging from 3 to 17 days, the rate of decline of the electrical gradient between the endolymph and perilymph was lower than that observed in control animals. Initial results indicated that the calculated K^+ conductance of the endolymph-perilymph barrier was 7 to 8.0 although different time course of K^+ conductance was consistently observed in noise exposed guinea pigs.

We plan (1) to continue to carry out this project as outlined and (2) to determine the possible correlation between the suppression of the sound induced responses and permeability changes in the noise-exposed animals and (3) to determine the electro-chemical profile of the hair cells by exploring the organ of Corti with ion selective electrodes.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is impossible at present to measure the permeability of the various cochler membranes directly and one useful indirect approach is to determine the diffusional alterations in the endolymphatic ionic concentration during anoxia. The present study will make a significant contribution to the understanding of noise-induced ear damage.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50042-01 LEB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Comparison of Impact Noise and Continous Noise Effects on Cochlear Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Teruzo Konishi	Medical Officer	LEB	NIEHS
	Alec N. Salt	Visiting Fellow	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Although the physiological effects of continuous noise on cochlear function are well documented in the literature, the effects arising from impact noise exposure have not yet been characterized. This is a direct consequence of the difficulty in generating and calibrating impact noise under laboratory conditions. A prototype impact noise generator has been used to test the widely accepted hypothesis that different types of noise of equal energy produce similar damage in the cochlea. Our results do not support the hypothesis.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Impact noise with peak sound pressures up to 132 dB sound pressure level (SPL) and a B duration of 29 msec was generated by a mechanical impact noise generator. Continuous broad band noise of equal energy to a given intensity of impact noise was generated by a loudspeaker system. Conscious guinea pigs with implanted round window electrodes, or anethetized guinea pigs with differential electrodes placed in the perilymphatic scalae, were exposed to a 20 minute period of impact noise or a similar period of broad band noise of equal energy. The suppression of tone induced responses (cochlea microphonics and action potentials) during and 1 hour following noise exposure was monitored by presenting 6 kHz test tones at 72 dB SPL.

MAJOR FINDINGS AND PROPOSED COURSE: Anesthetized guinea pigs were exposed to 20 minute periods of 132 dB peak SPL impact noise delivered at 1.2 impacts/second. During exposure the cochlear microphonics (CM) in response to test tones were suppressed to 24% of the pre-exposure value. The action potentials were not suppressed to the same extent, falling to 49.5% of the pre-exposure magnitude. During 1 hour recovery following exposure the cochlear microphonics (CM) and action potentials (AP) increased to 88.9% and 101.4% of their pre-exposure magnitudes respectively. The intensity of broad band noise necessary to deliver the same energy to a preparation during the 20 minute exposure period was 105 dBA. In guinea pigs exposed to this intensity of broad band noise the CM was only reduced to 80.5% of the pre-exposure value while the AP was in all cases totally abolished. During 1 hour recovery following exposure the CM and AP increased to 106.0% and 61.4% of their pre-exposure magnitudes respectively.

Restrained conscious guinea pigs exposed to the same levels of impact or continuous noise showed qualitatively similar results, the CM showing most suppression by impact noise and the AP showing most suppression by broad band continuous noise.

Our results indicate that the acute effects of continous and impact noises of equal energy are not equivalent. The concept that different types of noise of equal energy all produce similar amounts of damage to the cochlea must therefore be examined in greater detail.

The project will proceed in the following directions.

1. During the course of our experiment we noted that the frequency content of the impact noise affected the degree of response suppression observed, even when the noise energy was kept constant. This aspect will be further examined.

2. It has been reported that the combination of impact noise and continuous noise produces greater damage to the cochlea than either type of noise presented alone. This possibility will be investigated quantitatively.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The equal energy hypothesis has been widely employed in the development of noise regulations in Europe. Our results indicate that the measurement of energy may not be adequate to predict hearing loss in situations where impact noise is present. Further study of the relationships between hearing loss and the peak pressure, energy and frequency of impact noise are essential for the development of adequate noise regulations.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50043-01 LEB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effect of Noise on Embryo/Fetal Development in the Guinea Pig

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter Nawrot	Visiting Fellow	EBCB	NIEHS
	Reginald Cook	Acoustical Engineer	LEB	NIEHS

COOPERATING UNITS (if any)

Environmental Biology Branch, RRP, NIEHS

LAB/BRANCH

Laboratory of Environmental Biophysics

SECTION

Noise Effects Research Workgroup

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.75	.50	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

All laboratory studies on teratogenic potential of noise reported to date, have used the rat or mouse as the experimental animal. Yet the guinea pig is probably a more suitable experimental animal for such studies. In addition to endocrine similarity to man the audibility curve for the guinea pig is probably closer to that of the human than any other mammal except primates and chinchillas. The auditory sensitivity of rats or mice is such that the most sensitive frequency is nearly a decade above that of humans. While the guinea pig has been extensively used to investigate the effects of noise on the inner ear and on basic mechanisms of hearing the teratogenic potential of noise in the guinea pig has not been assessed.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS: Weaving room noise was selected as the exposure agent since it was utilized in the only study on the effects of noise on the guinea pig fetus and since it is experienced at high levels (105 dBA) by a significant number of women worldwide (in the hundred of thousands), and is a relatively broad band steady state noise which does not vary greatly worldwide. Mated females will be assigned randomly to one of four experimental groups; i.e. to a "Noise" group or to a "Control". Each group will contain at least 20 pregnant females. The guinea pigs in the "Noise" groups will be housed in an IAC animal exposure chamber from days 1 through 11 or from days 11 through 34 of gestation (postimplantation exposure). The days of exposure (1 through 11 and 11 through 34 of gestation) were chosen because implantation in the guinea pig takes place on day 6, but the connection with the uterine lumen is not lost until day 11 of gestation. Continuous weaving room noise will be presented at an intensity of 115 dBA for 8 hours daily. During the quiet period, the animals will be housed in a different chamber without the noise stimulus.

Body weights of all mated animals will be taken on days 1, 11, 34 and 35 of gestation. On day 35 of gestation, the guinea pigs will be coded before being transported to RTI where they will be sacrificed by CO_2 inhalation. Their reproductive status will be determined, the fetuses will be counted and then each will be examined for external, visceral and skeletal alterations.

The guinea pigs will be exposed to noise in an IAC animal exposure chamber modified for optimum sound field uniformity. The chamber provides ample protection from sound pentration through the walls. The data will be analyzed by the Mann-Whitney U-test with the litter considered as the experimental unit. A 5% probability level will be accepted.

MAJOR FINDINGS AND PROPOSED COURSE: The special acoustic chamber needed to efficiently carry out this experiment has been designed and ordered. The experiment will begin after the chamber is equipped with sound production equipment and problems relating to procurement of date bred guinea pigs have been resolved.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE INSTITUTE: Because of the rapid movement of females of childbearing age into occupations traditionally dominated by men, some of them very noisy, increasing concern has been expressed regarding possible general teratogenic and specific auditory risks to fetuses of mothers so exposed. The ability to predict risk factors for human exposure is to some degree related to the occurance of similar effects in different species and to the occurance of effects in species whose auditory systems more closely resemble those of humans. Use of guinea pigs rather than rats or mice satisfies both criteria.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50044-01 LEB

PERIOD COVERED
October 1, 1979 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effect of Different Noise Exposures on Embryo/fetal Development in the Mouse

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Reginald O. Cook	Acoustical Engineer	LEB	NIEHS
	Peter Nawrot	Visiting Fellow	EBB	NIEHS

COOPERATING UNITS (if any)
Environmental Biology Branch
Research Triangle Institute
Dept. of Nutrition, School of Public Health, University of North Carolina

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Noise Effects Research Workgroup

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.8	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Significant adverse effects on the course of embryo development have been reported in several mammalian species after exposure to high levels of "noise" during pregnancy in some, but not all studies conducted. An investigation was conducted to determine whether the characteristics of different noise exposure paradigms contributed significantly to this variability. Accordingly, three differing paradigms, thought to be representative of a wide range of noises, were devised to simulate (1) semi-continuous exposure to extremely high levels (126 dBA jet engine noise), (2) unanticipated high intensity starting sounds (microprocessor controlled pseudorandom onset, fast rise time alarm bells, sonalert type warning devices, or jet engine noise each at 112 dBA), (3) very high frequency sound to compensate for the upward frequency shift in rodents' hearing relative to humans (18-20 kHz modulated times at 110 dB from a commerical rodent repelling device). Pre- and post-implantation exposure to the first two paradigms resulted in decreased pregnancy maintenance and embryo lethality. Postimplantation exposure resulted in significantly increased fetolethality. No exposure related teratogenic corticosterone elevation effects found.

PROJECT DESCRIPTION

METHODS EMPLOYED: Mated female mice were exposed to noise in an Industrial Acoustics Corporation (IAC) double-walled audiometric room (4 x 3.5 x 3 m). The inside walls were lined with masonite for acoustical hardening purposes. The same chamber was also used for the quiet-environment control group. Ambient noise levels in the chamber before introduction of animals was less than 20 dB(A), as measured by a Bruel and Kjaer (B & K) type 2203 sound level meter. Because of noise generated by the animals themselves, Kimmel, et al. found that noise levels measured inside cages under similar conditions always exceeded 20 dBA. The housing conditions (temperature, humidity and light-cycle) were the same as provided in the animal quarters.

Sound amplification, control, and monitoring equipment were located outside the chamber; speakers and other sound producing equipment were suspended from a grid approximately one meter above the cages, which were located on the chamber floor.

Three noise exposure paradigms were used in this study. In the first experiment, sound was reproduced from a tape recording of extremely high intensity continuous jet engine noise obtained from the Aeromedical Research Laboratories, Wright Patterson AFB, Ohio. Although the levels produced by jet engine runup may exceed 140 dBA, this noise was reproduced at 127 dB(A), the highest level obtainable with the existing amplifier/speaker system. Cassette loop tapes were constructed from the master tape and each loop discarded after 12 hours.

To simulate conditions of random onset startling noise, an exposure paradigm involving microprocessor controlled pseudorandom presentation of three different sounds was employed in a second experiment. The three sounds were: (1) alarm bells at 110 dB(A). (2) a commercially marketed warning device called Sonalert, at 103 dB(A), and (3) a section of jet engine noise identical to that described previously, but reproduced at 110 dB(A). In order to insure that the animals would be exposed for at least a minimum period, the microprocessor was programmed to choose from one of 8 "on" periods (1,2,3,4,5,6,7, or 8 minutes) then from one of 8 "off" periods (9,11,13,15,17,19,21, or 45 minutes).

High frequency sound from a device commercially marketed for repelling feral rodents was used in the third exposure experiment. Sound from this device, called Rodo GardR, was presented at 113 dB(Linear). Rodo GardR did not produce the entire 18-20 kHz spectrum simultaneously, but swept downward from 20 to 18 kHz over a 3-second interval, then repeated the process after a 100 msec delay.

On Day 18 of gestation females of all experimental and control groups were sacrificed by cervical dislocation and their reproductive status was determined. The uterus was exposed and the number of implantation sites counted. The conceptus at each site was examined and classified as resorbed, dead, or alive. The full-term fetuses (live and dead) were individually weighed, the sex determined, and examined for external malformations. Any fetus weight 0.5 g or less or any live fetus weighing less than two-thirds the average of its largest litter mate was termed stunted. At least one-third of the fetuses of each litter were examined for visceral alterations. In addition, all stunted fetuses and those with external malformations were examined for visceral alterations. All live fetuses at time of sacrifice were processed and examined for skeletal alterations.

To investigate corticosterone levels in plasma of noise-exposed female mice, blood samples were obtained on days 1, 3 and 6 from experimental groups exposed to noise during days 1 through 6 of gestation. Blood was collected on days 6, 10 and 15 of pregnancy from experimental groups exposed during days 6 through 15 of gestation. Blood was obtained on days 1, 3, 6 and 15 of pregnancy from the control groups (animal quarters, chamber). At the onset at least 6 females from each experimental group were assigned randomly for blood collection on each designated day. To minimize the effects of diurnal variation in plasma corticosterone for all experimental groups, blood samples were always taken between 3:00 p.m. and 4:00 p.m. The animals were anesthetized by intraperitoneal injection of 8 ml/kg Nembutal and the blood samples taken from subclavian arteries directly into heparinized collect tubes by insiding the axillary region. To minimize any stressful effect on plasma corticosterone levels due to handling, blood collection was completed in less than 2 minutes.

Plasma corticosterone levels were measured by radioimmunoassay technique available from Endocrine Sciences (Tarzana, California) using rabbit antiserum obtained from Dr. G. Niswander (Fort Collins, Colorado) and radioactive 1,2#H-corticosterone (New England Nuclear Corporation). Assay for all samples were performed in duplicate and corticosterone values are expressed as mg/100 ml of plasma.

All experimental data were analyzed by nonparametric test procedures. The litter was considered as the experimental unit. Experimental groups were compared with controls by Mann-Whitney U test and Chi-square test. A 5% probability level was accepted.

MAJOR FINDINGS AND PROPOSED COURSE: Significant ($P < .05$) decreases in pregnancy rate were noted in all groups exposed to all noises except the group exposed to very high frequency noise post-implantation. Significant ($P < .05$) decreases in maternal and fetal weight gain occurred in the group exposed pre-implantation to high intensity startling sounds. Significant ($P < .05$) embryolethal effects occurred in the group exposed pre-implantation to the extremely high intensity jet noise paradigm, and significant fetolethal effects were associated with post-implantation exposure to the very high frequency noise paradigm. No significant teratogenic effects or corticosterone alterations were noted.

Because the increase in fetolethality resulting from post-implantation exposure to the high frequency sound was both dramatic and unique, it appeared to provide a model from which hormonal/biochemical alterations might be determined. Since the introduction of exogenous catacholamines has been reported to induce date stage fetolethality, the exposure is to be replicated and catecholamine levels measured.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Because of the rapid movement of females of childbearing age into occupations traditionally dominated by men, some of them very noisy, increasing concern has been voiced regarding possible general teratogenic and specific auditory risks to fetuses of mothers so exposed. Several epidemiological studies around airports have reported low level increases in teratogenicity. Our findings indicate that at least in the mouse, teratogenicity resulting from excessive noise exposure is very difficult to induce. Other reproductive aberrations appear to be much more likely.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50045-01 LEB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Relationship of Catecholamine Levels and Fetolethality in Noise Exposed Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Reginald O. Cook	Acoustical Engineer	LEB NIEHS
	Peter Nawrot	Visiting Fellow	EBCB NIEHS

COOPERATING UNITS (if any)

Research Triangle Institute
Environmental Biology and Chemistry Branch

LAB/BRANCH

Laboratory of Environmental Biophysics

SECTION

Noise Effects Research Workgroup

INSTITUTE AND LOCATION

NIEHS,NIH, P.O. Box 12233, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.4	.3	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Since a previous experiment revealed that late stage pregnancy exposure of CF-1 mice to high frequency noise resulted in a significant increase in fetolethality, the hormonal/biochemical correlates of this effect are to be sought. Since corticosterone levels were measured and found to be unaffected by noise exposure, this experiment will focus on catecholamines, because exogenous introduction of these substances has been found to increase fetolethality. Exposure period will be 12 hours (noon to midnight) as before.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Separate groups of pregnant mice will be exposed to pre- and post-implantation high frequency noise (18-20 kHz) at 110 dB sound pressure level and their concept examined for teratogenic and embryo-fetotoxic effects.

In addition, plasma catecholamines (epinepherine, norepinepherine, dopamine) will be assayed by a radioenzymatic-paper chromatographic technique described by Weise and Koper. Present plans are to have this accomplished by contract.

MAJOR FINDINGS AND PROPOSED COURSE: In previous experiments it was shown that exposure of pregnant female CF-1 mice to noise located in the most sensitive portion of their audibility curve resulted in a significant increase in fetolethality. This effect occurred around 20 kHz. The purpose of this experiment is to reconfirm the previous data and to determine the biochemical correlates of this result. Corticosterone levels measured in the initial study showed no significant increase associated with noise exposure.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Identification of hormonal/biochemical correlates of nonauditory effects of noise is a necessary step to understanding these effects sufficiently for predictive purposes. Since previous work showed high frequency noise to produce unique effects, replication is also important.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50046-01 LEB

PERIOD COVERED
January 1, 1979, September 30, 1979

TITLE OF PROJECT (80 characters or less)
Mechanisms of Chemically Induced Photosensitivity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Colin F. Chignell	Chief	LEB	NIEHS
OTHERS:	B. Kalyanaraman	Visiting Fellow	LEB	NIEHS
	R. P. Mason	Research Chemist	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.1	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to determine the role played by light-induced free radicals in chemically induced skin photosensitivity. Free radicals have been detected during light irradiation of 4-aminobenzene-sulfonamide (I), 4-nitrobenzenesulfonamide, 4-nitroaniline, 4-aminobenzoic acid (II) and 4-nitrobenzoic acid. This finding may help to explain the phototoxic and photoallergic properties of I and II.

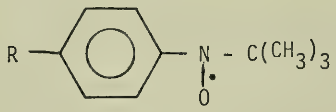
PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Many chemicals are know to cause photosensitivity in certain individuals. The photosensitive response may be one of two types, either phototoxic or photoallergic. The phototoxic reaction generally occurs during a subject's first exposure to light after the administration or topical application of a chemical and usually takes the form of an exaggerated erythemal response ("sunburn"). Photoallergic individuals may also exhibit an initial erythemal reaction. As this subsides, delayed abnormal responses begin to appear including popular, eczematous and unticovial reactions. Such reactions to light may persist for months after avoidance of the photoallergen.

While the initial step in all forms of photosensitivity must be the absorption of light by the chemical or its metabolites, the precise mechanism of photoxicity and photoallergy is unknown. In this study we have employed spin-trapping agents (2-methyl-2-nitrosopropane) to detect the formation of free radicals during the light irradiation of substituted aromatic compounds. The structure of the trapped radicals, and the initiating free radicals has been determined with the aid of electron spin resonance.

MAJOR FINDINGS AND PROPOSED COURSE: Free radicals were generated during the light irradiation of the following compounds: 4-aminobenzenesulfonamide (I) (sulfanilamide), 4-nitrobenzenesulfonamide (II), 4-nitroaniline (III), 4-aminobenzoic acid (IV), 4-nitrobenzoic acid (V). The structure of the free radicals was found to be



where R = SO_2NH_2 (I + II), R = NO_2 (III), R = COOH (IV) and R = NO_2 or COOH (V). No free radicals could be detected during light irradiation of aniline, benzoic acid and benzenesulfonamide. These findings suggest that the photoallergic properties of I and IV may be due to the formation of a free radical in the skin of susceptible individuals.

These studies will be extended to other aromatic molecules, including those found in effluents from energy-related processes, that are known to cause photosensitivity. Evidence will also be sought for other possible mechanisms including smglet oxygen formation ("Photodynamic" effect) and energy transfer to biologically important macromolecules.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many drugs and other environmental chemicals are known to cause skin photosensitization. These studies will help to provide a molecular basis for understanding this toxic effect. When the mechanism of photosensitization is know it may be possible to design a test which will identify those chemicals which could have potentially harmful effects on the skin in the presence of light.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50047-01 LEB

PERIOD COVERED
November 1, 1978, to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Biological Effects of Fluorescent Lighting

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Colin F. Chignell	Chief	LEB	NIEHS
	Donald D. Feldman	Veterinarian	CMB	NIEHS
OTHER:	Hugh Tilson	Research Pharmacologist	LBNT	NIEHS

COOPERATING UNITS (if any)
Comparative Medicine Branch, RRP, NIEHS
Laboratory of Behavioral and Neurological Toxicity

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to determine whether artificial lights have any biological effects. The effect of three different fluorescent lighting systems (simulated daylight, coolwhite, pink) on the C_3H mouse are being studied. The parameters measured include longevity, tumor incidence, reproduction (litter size, sex ratio etc.), pathology and behavior.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The purpose of this study is to determine the biological effects of fluorescent lighting on the C_3H mouse. This strain was selected because it spontaneously develops mammary tumors and because previous studies have suggested that exposure to certain fluorescent lighting decreases lifespan. Three types of fluorescent lights were selected for this work: cool white (GE F40CW), daylight (GE F40C50) and pink (GE F40PK). The lights were kept on for 12 hrs daily (6:00AM-6:00PM). Sexually mature female mice were allowed to conceive under the cool white light and then were transferred to the appropriate light environment for delivery. After weaning the mice were paired and kept under the same lighting conditions.

MAJOR FINDINGS AND PROPOSED COURSE: At the present time no difference have been found in mice kept under the three different lighting systems. The experiment will be continued and the following parameters studied: longevity, tumor incidence, reproduction (litter size, sex ratio etc.) pathology, muscle strength and behavior. In addition to the mated pairs, separate groups of male and female mice will be reared under the fluorescent lights to determine the effects of non-breeding.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Fluorescent lights are more efficient than conventional incandescent lights. However the energy spectrum of fluorescent lighting is considerably different from that of natural daylight. It therefore is important to know whether the distorted spectrum of fluorescent lights produces any undersirable biological effects.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50048-01 LEB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Characterization of Lung Lamellar Bodies Using Spin Labels

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Mary J. Ortner	Staff Fellow	LEB	NIEHS
	Gary E.R. Hook	Senior Staff Fellow	LPFT	NIEHS
	Judson Spalding	Research Biologist	LPFT	NIEHS

COOPERATING UNITS (if any)

Laboratory of Pulmonary Function and Toxicology

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.3	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An electron spin resonance probe (5-doxyl methyl stearate) has been used to characterize the fluidity of lung Type II cell lamellar bodies which store pulmonary surfactant. These lecithin containing multilaminate structures were considerably less rigid than erythrocyte ghosts or normal leukocyte membranes, and were not changed by freezing or sonication. The lamellar bodies showed a temperature-dependent fluidity profile which was identical to liposomes made from lamellar body - extracted lipids; however it differed significantly from liposomes made of pure dipalmitoyl lecithin (DPL). Furthermore, liposomes made from a DPL-phosphatidyl glycerol combination which closely resembled the major lamellar body phospholipid components, were significantly different from the natural lamellar body liposomes. This indicates that perhaps minor phospholipids may play an important role in determining the molecular order maintained within the lamellar bodies.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The properties of lamellar bodies from rabbit lung type II cells have been investigated using electron spin resonance (ESR) probes. The lamellar bodies were extracted and purified according to well established procedures. The spin label (5-doxyl methyl stearate) was purchased from Synva Associates, Palo Alto, California. ESR measurements were taken using a Varian E-109 X-band spectrometer equipped with a E-238 Tm_{110} cavity and a variable temperature apparatus.

MAJOR FINDINGS AND PROPOSED COURSE: 1. Artificial Systems. The effect of temperature on the motion of 5-doxyl methyl stearate (5 DMS) in liposomes made from pure dipalmitoyl lecithin (DPL) was investigated. At high temperature (52°C - 41°C) the probe reported an almost constant fluidity; however between 41°C and 38°C a sharp transition point occurred. The increase in fluidity of the probe then progressed in a linear manner as far as 22°C. Liposomes made from egg phosphatidyl choline also showed a similar transition temperature; however the increase in fluidity as a function of temperature was much less than the DPL liposomes. We have also examined phosphatidyl glycerol ((PG) which constitutes about 12% of the lamellar body phospholipids) and DPL/PG combination liposomes. The PG liposomes bound the spin label in a more highly immobilized manner than the DPL liposomes at temperatures between 45°C-21°C and the combination liposomes were intermediate. 2. Lamellar Bodies. The effect of temperature on the motion of 5-DMS in extracted lamellar bodies was studied. The probe showed a fluidity in the lamellar bodies which was closer to the artificial systems and much higher than human erythrocyte ghosts or normal leukocyte membranes. The effect of temperature showed a slight transition point at 40°C and an additional transition at 28°C. Lamellar bodies which had been sonicated or frozen were similar to the native state organelle. In further experiments, mixed lipids were extracted from the lamellar bodies using chloroform/methanol (2:1), and liposomes were formed. The motion of the spin label in the liposomes was unchanged from that of the native state lamellar bodies at temperatures between 50°C-22°C.

Although the motion of the spin label in the lamellar bodies was closer to that of the artificial systems than that of ghost or leukocyte membranes, it was significantly different from the artificial liposomes composed of the major constituent phospholipids (DPL/PG). This finding indicates that perhaps a minor lipid component may be influencing the structure of the lamellar bodies.

These spin probe studies are being currently expanded to study other artificial systems including some of the minor lipid components of the lamellar bodies. We hope to thereby eventually duplicate the natural fluidity profile and thereby to determine the effect of minor components of the lamellar bodies. In addition, we are currently isolating sufficient quantities of lamellar bodies to isolate and separate the component lipids to determine the effect of each on the temperature profile of the intercalated spin label. When these studies are completed the effect of exposure to oxidizing gases (SOx, NOx) on the molecular structure of the lung lamellar bodies will be examined.

SIGNIGICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The acellular layer of surfactant which is essential for normal pulmonary function is stored by the lamellar bodies of Type II cells. These lecithin-rich organelles also contain hydrolytic enzymes and may be a source of the hydrolases secreted into the acellular lining of the branchiotres. Although the lipid components of these airways have been isolated and characterized, there has been no thorough study of the interaction of these components with one another within the lamellar body. This study should provide information leading to a more complete characterization of the molecular structure of this organelle which is essential to normal pulmonary function.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50049-01 LEB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Spin-Trapping and Direct ESR Studies of Anticancer Quinone Metabolites

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Kalyanaraman	Visiting Fellow	LEB	NIEHS
OTHERS:	Ronald P. Mason	Research Chemist	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Spin-trapping of the superoxide free radical has provided ESR evidence of the formation of this species in microsomal incubations of adriamycin, daunorubicin, and mitomycin C. The time sequence of the appearance of the spin-trapped superoxide and the semiquinone radical metabolite of these quinone-containing anticancer drugs indicates that air oxidation of the semiquinone metabolite is responsible for the superoxide formation. Superoxide dismutase prevents the formation of the superoxide spin adducts. Microsomal incubations containing DNA-intercalated anthracyclines produce much less superoxide than incubations without DNA. The first clear ESR evidence for the semiquinone metabolite of mitomycin C in a biological system is presented.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: ESR measurements were made with a Varian century series E109 spectrometer equipped with a TM_{110} cavity. gValue measurements were done with an E-232 dual sample cavity using Fremy's salt as a g-standard ($g = 2.0055$).

MAJOR FINDINGS AND PROPOSED COURSE: In our study we have unambiguously observed the mitomycin C semiquinone in anaerobic microsomal incubations and the spin-trapped superoxide in similar aerobic incubations. In addition, we report direct ESR and spin-trapping evidence suggesting that the autoxidation of the mitomycin C semiquinone and the anthracycline semiquinone free radicals, in fact does result in the formation of superoxide. The spin-trapped superoxide ESR spectrum is characterized by known hyperfine couplings, so the identity of this species is not dependent upon the activity of superoxide dismutase, as was the case in the earlier investigations.

At present two free radical mechanisms for the cytotoxic anticancer quinone compounds have been proposed. Evidence has been presented suggesting that the formation of the semiquinone is rate-determining in the covalent binding of mitomycin C to DNA, and it has been suggested that the semiquinone of these antitumor compounds are sufficiently stable to enter the nucleus, and either to intercalate into or react with DNA, or other macromolecules as a consequence of the affinity characteristics of these compounds.

The second mechanism of biochemical activation, for which considerable support has been established, focuses instead on the quinone-catalyzed superoxide or superoxide-derived species. Our work emphasizes that these two mechanisms can not be operating simultaneously, because superoxide generation necessarily destroys the semiquinone. Poorly perfused hypoxic tumors are possibly an important exception to this argument, because such tissues will have much lower levels of intracellular oxygen.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Quinone compounds are very commonly used both in industry and medicine, as antibacterials and anticancer drugs. The redox metabolism of these compounds is thought to be of importance in their mode of biological activities. This redox mediated toxicity is not limited to bacterial or neoplastic cells but should effect all metabolically active cells.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50050-01 LEB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

ESR Evidence for a Free Radical in the Cis-Trans Isomerization of Furylfuramide

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ronald P. Mason	Research Chemist	LEB	NIEHS
Others:	B. Kalyanaraman	Visiting Fellow	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Environmental Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It has been proposed that the enzymatic cis-trans isomerization of furylfuramide is the result of anion free radical formation by nitroreductases. Electron spin resonance measurements of the furylfuramide anion free radical have provided direct spectral evidence for this intermediate, and clarified the disputed relationship between the isomerization and the nitro reduction of furylfuramide.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: ESR spectra of anaerobic microsomal incubations at 25° were obtained with a Varian century series E-109 spectrometer equipped with a TM$_{110}$ cavity. Visible spectra of *cis*- and *trans*- furylfuramide (AF-2) were obtained with a DW-2A Aminco-Chance spectrophotometer at 37°.

MAJOR FINDINGS AND PROPOSED COURSE: Tatsumi *et al.* have proposed that enzymatic *cis-trans* isomerization of furylfuramide is a direct consequence of enzymatic nitro reduction. The nitroreductases, which are inhibited by oxygen, transfer a single electron to nitro substrates to give their respective anion free radicals. The carbon-carbon double bond linking the two furan rings of AF-2 would be weakened by anion radical formation, because the additional electron is in an antibonding molecular orbital. Upon formation, the *cis*-AF-2 anion free radical was proposed to isomerize rapidly to the *trans*-AF-2 anion, which could then be oxidized to form *trans*-AF-2.

Recently, Tomoeda and Kitamura studied the isomerization and nitro reduction of *cis*-AF-2 by subcellular fractions of *E. coli*. Their observations suggested that the nitro reducing and isomerizing activities of E. coli were due to totally different enzymes, in apparent contradiction of the isomerization mechanism of Tatsumi *et al.*

The ESR spectrum of an anaerobic microsomal incubation containing furylfuramide and an NADPH-generating system provides direct evidence of free radical formation. In the presence of air, nitroaromatic anion free radicals undergo rapid air oxidation to form superoxide anion, and the nitroaromatic anion free radicals are not detected. In addition, identical spectra were obtained with either *cis*- or *trans*-AF-2. These ESR spectra did not vary with time even though visible spectroscopy shows a rapid and nearly complete conversion of *cis*-AF-2 to *trans*-AF-2.

Although spectral evidence alone indicates that both the major and the minor components of the spectrum represent AF-2 anion free radicals, the assignment of the spectrum to *cis-trans* anion free radicals must be made on other grounds. Preliminary INDO (intermediate neglect of differential overlap) molecular orbital calculations on *cis*- and *trans*-AF-2 anions suggest that the *cis* anion radical will have the larger nitrogen hyperfine splitting constant. Furthermore, the greater steric hindrance expected for the *cis*-conformer should result in a predominance of the *trans*-conformer. In the parent compounds, the greater thermodynamic stability of *trans*-AF-2 is clearly indicated by the relative conversion of *cis*-AF-2 to *trans*-AF-2 (87-91%) vs that of *trans*-AF-2 to *cis*-AF-2 (7-11%) observed in xanthine oxidase incubations under optimum conditions. Clearly, since xanthine oxidase is only a catalyst, and cannot change the thermodynamic equilibrium of *cis*- *trans*-AF-2, the equilibrium ratio of *cis* to *trans* is approximately 10:90. The similarity in the equilibrium ratios of *cis*- to *trans*-AF-2 and the two free radicals is the strongest chemical evidence for our assignment of the two species as the respective anion radicals. The observation of distinct conformational isomers with ESR only requires that the equilibrium mixture be slowly interconverting on the ESR time scale, which is very rapid. More precisely, the lifetime of the conformers must be much longer than $1/\gamma_e\ (a^N_{cis} - a^N_{trans}) = 28$ nanoseconds. This reaction can still be fast in terms of chemical reaction, and in fact, competes favorably with the autoxidation of the AF-2 anion free radicals.

The results of Tomoeda and Kitamura and the isomerization mechanism of Tatsumi *et al.* are not contradictory if the oxygen-insensitive *E. coli* reducing activity does not form the anion radical intermediate and the oxygen-sensitive *E. coli* reducing activity does form the anion radical, as has been recently demonstrated. In the present study we provide electron spin resonance evidence for *cis*- and *trans*-AF-2 anion radical intermediate formation during the microsomal *cis-trans* isomerization of AF-2. This study is now completed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Furylfuramide has been shown to be mutagenic, carcinogenic and cytotoxic. Enzymatic nitro reduction of furylfuramide is thought to form the reactive metabolites which are primarily responsible for these effects. The synthetic form of this former food additive is the *cis* isomer. Typical mammalian nitroreductases, such as xanthine oxidase or rat liver microsomal nitroreductase, isomerize *cis*-furylfuramide to *trans*-furylfuramide before they initiate reductive "activation". Many investigators have observed this isomerization in the course of investigations of the reductive activation of *cis*-AF-2 by mammalian and bacterial systems.

PUBLICATIONS

Electron Spin Resonance Evidence for Free Radical Intermediate in the *Cis*-Trans Isomerization of Furylfuramide, Molecular Pharmacology (in press)
B. Kalyanaraman, Edward Perez-Reyes and Ronald P. Mason.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50051-01 LEB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Free Radical Metabolism of Polycyano Compounds

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ronald P. Mason	Research Chemist	LEB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Polycyano compounds have been proposed for use as superconductors in high voltage transmission lines. These compounds are known to be strong electron acceptors which also form charge-transfer complexes. It is the objective of this study to examine the biological properties of polycyano compounds and to determine their metabolic fate. Preliminary experiments have shown that in microsomal incubations the electron transfer between tetracyanobenzene and some unknown biological donor is complete and the radical anion of this compound is formed. The electron transfer to form the tetracyanobenzene anion radical is dependent upon the presence of NADPH. In future studies the source of the electron will be sought and the microsomal metabolism of other polycyano compounds will be examined.

PROJECT DESCRIPTION

METHODS EMPLOYED: Electron spin resonance (ESR) spectroscopy was employed to detect steady-state levels of free radicals.

MAJOR FINDINGS AND PROPOSED COURSE: Tetracyanobenzene was metabolized to an anion free radical by rat liver microsomes. The ESR spectrum of this free radical metabolite was dependent upon NADPH. We now intend to determine what other metabolites are formed by this reduction path, because microsomal incubations acquire a pink color due to a metabolite, which is not a free radical. Cyanide is also expected to be a reduction metabolite, because it could form by reductive cleavage of the phenyl-nitrile bond.

Next, we will investigate the source of the single electron which was transferred to tetracyanobenzene. The main possibilities are cytochrome P-450 and b_5 and their respective flavin-containing reductases, NADPH-cytochrome P-450 and NADPH cytochrome b_5 reductase.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Tetracyano-compounds are used in organic superconductors, which are thought to have great potential in the transmission of high voltages without power loss. Although these compounds are reported to have fungicidal and bactericidal properties there is little or no information on their other biological effects and nothing is known about their toxicity and metabolic fate.

PUBLICATIONS

Mason, R.P.: Free radical metabolites of foreign compounds and their toxicological significance. Hodgson, Bend, Philpot (Ed.): Reviews in Biochemical Toxicology. Elsevier North Holland, Inc., 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50052-01 LEB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Binding of Chemically Activated Semiquinone Free Radicals from Anticancer Agents to DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Birandra K. Sinha	Senior Staff Fellow	LEB	NIEHS
OTHERS:	Colin F. Chignell	Chief	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Chemical reduction of the highly active quinone-containing antitumor drugs, adriamycin and daunorubicin formed the same partially reduced free radical previously reported by microsomal activation. *In vitro* incubation of the chemically activated free radical intermediates with DNA resulted in covalent binding of these drugs to DNA. The adriamycin semiquinone radical has a greater affinity for DNA and covalent complexes containing up to one adriamycin per 15 nucleotides were obtained. The daunorubicin semiquinone radical, on the other hand, showed a lesser binding affinity and gave rise to complexes in which one drug molecule was covalently bound per 140 nucleotides. The stronger covalent binding of adriamyci to DNA may account for more severe DNA damage induced by this drug.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The anthracycline antitumor drugs, adriamycin and daunorubicin, both of which contain the quinone moiety, are currently being used in the treatment of acute leukemia and solid tumors in man. In addition, these agents induce damage in DNA as evidenced by an increase both in chromosomal aberrations and in the frequency of sister chromatid exchange (SCE). The biological activities of these agents seem to result from their ability to intercalate into DNA base pairs by virtue of their planar ring structure. Such tight binding to nucleic acids results in the inhibition of both DNA and RNA synthesis and DNA- and RNA- dependent polymerase. Handa and Sato have shown that the anthracycline antitumor drugs, mitomycin- C, daunorubicin and adriamycin stimulate the formation of superoxide in microsomal incubations containing NADPH.

Adriamycin (1 mg/ml), daunorubicin (1 mg/ml) and mitomycin-C (2 mg/ml) were dissolved in 5 mM phosphate buffer (pH 7.4) and were deaerated by bubbling nitrogen for 10 min. Chemical activation was carried out by adding in 5 equal portions, at 5 min intervals, sodium borohydride solution (2 mg/ml in phosphate buffer, 0.75 equivalent of $NaBH_4$ added) to adriamycin and daunorubicin solutions. Calf thymus DNA (1 mg/ml), dissolved in 5 mM phosphate buffer (pH 7.4) containing 50 mM NaCl, was deaerated by bubbling nitrogen for 10 min. The DNA solution was added (1 mg DNA/1mg drug) to the activated adriamycin and daunorubicin and the mixtures were incubated in the dark for 18 hrs at room temperature. The DNA-drug complexes were isolated by ethanol precipitation and the unbound drugs were removed. The complexes were washed 3 times with ethanol (assay for total bound drugs were carried out on these samples). The DNA-drug complexes were dissolved in 2 M NaCl solution to dissociate intercalated drugs, stirred for 1 hr and reprecipitated in ethanol. After the dissociated drugs were removed, the complexes were redissolved in 2 M NaCl and dialyzed extensively against 5mM phosphate buffer containing 1 M NaCl. The DNA-drug complexes were then chromatographed in Sephadex G-100 column (2.0 x 30 cms) using 5 mM phosphate containing 100 mM NaCl as eluent. Activation of mitomycin-C was achieved by mixing DNA (2 mg DNA/1 mg drug) with mitomycin-C, and adding the reducing agent in 5 equal portions at 5 min intervals as described by Tomasz. The DNA-mitomycin-C complex was isolated as described for adriamycin and daunorubicin.The assays of the binding ratios in the DNA-drug complexes were carried out spectrophotometrically. The binding ratio is defined here as the molar ratio of mononucleotide unit to the drug. Thermal denaturation studies were carried out according to previously published methods. The stability of the DNA-drug complexes was demonstrated by treating the complex with sodium dodecyl sulfate (SDS) and urea, agents that are known to dissociate noncovalent complexes, and monitoring loss of any bound drug spectrophotometrically.

MAJOR FINDINGS AND PROPOSED COURSE: The formation of semiquinone radical intermediates from quinone-containing antitumor drugs in microsomal incubation is well established. Lown *et al*. and Tomasz *et al*. have shown that chemical reduction of the mitomycins leads to the formation of the semiquinone radical intermediate which binds covalently to DNA. Under our conditions of chemical activation and using Fremy's salt as standard, the g-values for adriamycin and daunorubicin semiquinone radicals were found to be 2.0035 and 2.0037 respectively, which are similar to those reported by Sato suggesting that the same free radical intermediates are formed from either microsomal or chemical activation. The partially resolved ESR spectra of daunorubicin and adriamycin result from the hyperfine splittings of aromatic hydrogens. Addition of DNA to daunorubicin and adriamycin semiquinone radicals resulted in loss of the hyperfine structure suggesting that these semiquinone radicals become immobilized upon binding to DNA.

Bachur _et al_. have proposed that anthracycline semiquinone radicals formed by microsomal incubation may interact selectively with nucleic acids. Tomasz _et al_ have shown that chemically activated mitomycin semiquinone radicals bind covalently to DNA and other polynucleotides. Table 1 shows the binding ratios for chemically activated semiquinone free radicals from adriamycin and daunorubicin. We have included the binding ratio for mitomycin-C obtained under our experimental conditions for comparative purposes, and it is similar to the one obtained by Tomasz. The results in Table 1 clearly show that adriamycin and daunorubicin, in addition to intercalating into DNA, are bound covalently to the nucleic acid. The striking difference in both the total binding ratios and the covalent binding ratios (Table 1) between two similar compounds, adriamycin and daunorubicin, is very surprising. Covalent binding of adriamycin to DNA may account for the ability of this drug to induce extensive chromosomsal aberration and increase the frequency of SCE. It is interesting to note that mitomycin-C, another strong inducer of SCE also exhibited a binding ratio similar to that observed with adriamycin (Table 1). The covalent binding of adriamycin and daunorubicin to DNA could then explain why there is an absolute requirement for cellular activation before _in vivo_ DNA strand breaks are observed. Furthermore, the higher covalent binding of adriamycin to DNA could also explain its more severe and progressively increased damage to DNA.

The concept of covalent binding to DNA was established by carrying out studies under a variety of conditions known to dissociate noncovalent DNA-drug complexes. Muller and Crothers have shown that sodium dodecyl sulfate (SDS) dissociates actinomycin -DNA complexes. Furthermore, SDS (0.1-.2%) has also been used to dissociate daunorubicin -DNA complexes. Urea dissociates actinomycin from DNA and high ionic strength is also known to break noncovalent drug-DNA complexes. The drug -DNA complexes of adriamycin and daunorubicin isolated in this investigation were found to be stable to the following conditions (a) 5% SDS, 3 hrs, (b) 7M urea, 40 min, (c) 2M NaCl; 1 hr and (d) pH 12.0, 15 min. Similar stabilities have been shown for covalent complexes of mitomycin-C with DNA. Thermal denaturation data with adriamycin- and daunorubicin- DNA complexes are presented in Table 2. The melting profiles of the complexes were broad. An increase in T_m associated with a broader melting profile is characteristic of covalent binding. The small increase of

1°C obtained with the daunorubicin -DNA complex may be due to small amount of this drug that is covalently bound to DNA. Since adriamycin- and daunorubicin- DNA complexes remained stable under conditions known to dissociate intercalated complexes and exhibited a characteristic broad melting profile only seen with covalently bound drugs, we conclude that the semiquinone radical intermediates bind covalently to DNA. In addition to covalent binding, intercalation of these intermediates into DNA base pairs is also involved, as 40% of adriamycin and 80% of daunorubicin were found to be intercalated. Covalent binding must follow the intercalation of the drug semiquinone radicals into DNA.

Future plans include characterization of the binding sites of these drugs in DNA by a) using synthetic biopolymers and b) selective degradation of the DNA-drug complexes.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since the mechanism of action of antitumor drugs and chemical carcinogens stems from their ability to interact with nucleic acids, it is of great significance to understand such binding at a molecular level. It is hoped that by defining these interactions, a better understanding of the tumorigenicity will result.

TABLE 1

BINDING OF SEMIQUINONE RADICALS TO DNA

Drug	Total binding ratio[a]	Covalent binding ratio[b]	SCE[d]/Cell
Adriamycin	10	15	33
Daunorubicin	30	140	18
Mitomycin-C	10	12	99.5
Adriamycin[c]	10	75	-

---

[a] Total binding ratios (mole nucleotide/mole drug bound) were obtained by analyzing drug - DNA complexes after ethanol precipitation.

[b] Covalent binding ratios (mole nucleotide/mole drug bound) were obtained by dissolving the drug - DNA complexes in 2 M NaCl and performing extensive dialysis, followed by chromatographic isolation using Sephadex G-100.

[c] Adriamycin was reduced to hydroquinone in one step by adding the reducing agent.

[d] Sister chromatid exchange; adriamycin and daunorubicin at doses 12 mg/kg and mitomycin C at 5 mg/kg, in bone marrow cells *in vivo*. Controls had SCE of 5.

TABLE 2

T_m DETERMINATION[a]

	T_m, (°C)	ΔT_m, (°C)
DNA	68.5	-
DNA-adriamycin[b]	83.5	15.0
DNA-daunorubicin[c]	69.5	1.0

[a] T_m determinations were carried out in 5 mM phosphate buffer (pH 7.4) at 260 nm by means of a Gilford 250 recording spectrophotometer with a thermoprogrammer 2527 programmed for a temperature rise of 1.0°C min.

[b] Binding ratio 1 mole drug/15 moles nucleotide.

[c] Binding ratio 1 mole drug/140 moles nucleotide.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50053-01 LEB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Binding of Antitumor Drugs to Membranes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Birandra K. Sinha	Senior Staff Fellow	LEB	NIEHS
OTHERS:	Mary J. Ortner	Staff Fellow	LEB	NIEHS
	Colin F. Chignell	Chief	LEB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The cytotoxic and mutagenic properties of antitumor drugs such as adriamycin, acridines, diacridine, actinomycin D and Pt compounds are related to their interaction with nucleic acids and inhibition of protein synthesis. We have examined their interaction with human erythrocyte ghost membranes and murine mastocytoma cells using spin labeling techniques. These drugs induce changes in electron spin resonance of the spin labeled ghost membranes and in the mastocytoma cells. These findings suggest that these drugs induce changes in protein conformation of the membranes. The membrane binding properties of these drugs may be important in their mechanism of action.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The antitumor activities of anthracycline antibiotics (adriamycin, daunomycin), acridines (diacridine, AMSA), actinomycin D and Pt-compounds are believed to result from their interactions with DNA and consequent inhibition of DNA and RNA synthesis. While the intercalative binding of these drugs to DNA is important for their cytotoxic and mutagenic actions, the molecular mechanism(s) of action remains poorly understood. Recent studies of Murphee et al. have shown that anthracyclines such as adriamycin, a strong intercalator to DNA, bind to the membrane of Sarcoma 180 ascites cells in a concanavalin A-mediated agglutination assay. Diacridines, prepared as antitumor agents due to their high affinity towards nucleic acids, have also been shown to bind with membranes. Fico et al. have shown that diacridines exhibit a significant inverse correlation between in vivo percent increase in life span (ILS%) and their ability to enhance the rate of agglutination of S-180 cells. Furthermore, ILS% of diacridines did not correlate with inhibition of nucleic acids synthesis. These studies suggest that antitumor effectiveness may be associated with some mechanism of action other than inhibition of nucleic acid synthesis.

MAJOR FINDINGS AND PROPOSED COURSE: Human erythrocyte ghost membranes and mouse P-815 mastocytoma cells were spin labeled with a maleimide spin label (MSL). Samples for ESR studies were prepared by the addition of a methanolic solution ($1x10^{-2}$M) of drugs (except the Pt-compound which was dissolved in water) to the spin labeled ghosts in 5 mM phosphate (pH 7.4) and to the spin labeled cells in Lockes solution (pH 7.4). The controls contained equivalent amounts of methanol. The drugs were incubated with spin labeled ghosts for 18 hrs at 4°C and warmed to room temperature before ESR spectra were recorded. The drugs were incubated with P 815 cells for 30 min at room temperature. The ESR spectra were recorded with a Varian E109 spectrometer operating at 100 kHz. The samples were introduced into an E-238 TM_{110} cavity in quartz flat cells. The reaction of MSL with sulfhydryl groups of proteins and erythrocyte membranes is well established. The ESR spectrum of MSL labeled ghost membranes is similar to that obtained by others. Such a spectrum is a composite of two spectra; one due to a strongly-immbolized radicals(S) and the other due to a weakly-immobilized(W) radicals. The ratio of the amplitudes of the two peaks (W/S) was taken as a relative amount of two conformations of the proteins present in the membranes.

The interaction of the antitumor drugs with the ghost membranes was followed by observing the ESR spectrum of the nitroxide radical and the data is presented in Table 1. The results clearly indicate that a small (5-15%) but significant change in protein conformation occurred due to an interaction with the drugs. Sandberg, et al. have suggested that the strongly immobilized spectrum results from the labeling of proteins located within the lipoprotein matrix of the membrane. The weakly-immobilized spectrum, by contrast, is probably associated with labels that are in a polar environment on the membrane surface. The data in Table 1 show that all the antitumor drugs (except diacridine at higher concentration) cause a conversion of the strongly immobilized labels to weakly immobilized labels. This suggests that these drugs cause a change in the conformation of labels that are buried within the lipoprotein matrix. The diacridine also causes a conformational change which appears to be associated with protein that are located at the surface. The ESR spectrum of MSL-labeled mastocytoma cells is similar to that observed with labeled ghost membranes. The interaction of the antitumor drugs with these cells was similarly followed by observing the ESR spectrum and the changes in W/S ratio are presented in Table 2. The results suggest that the antitumor drugs interact with membrane proteins in a similar fashion to that seen with the ghost membranes. In both systems, the cis-Pt compound and AMSA bring about the largest changes (15%) suggesting a stronger interaction with membrane proteins. It seems possible that the antitumor drugs may have a similar site on both human ghost membranes and the mastocytoma cell membranes. It is of interest that the interaction of these drugs is different from that of the phenothiazines which cause a conversion of weakly immobilized label to strongly immobilized labels. Conformational changes in the membrane proteins induced by drugs are known to inhibit physiologically significant functions of erythrocyte membranes, such as glucose and ion transport. Thus, it appears possible that antitumor drugs may exert their biological effects not only by interaction with DNA but also by modifying cell membrane function.

Work is in progress on the binding of anthracycline antitumor drugs (adriamycin and daunorubicin) to human ghost membranes and mastocytoma cells using fluorescence microscopy. Preliminary studies with these drugs suggest that there are at least two different binding sites in the mastocytoma cells (inside the cells and outer membranes of the cells). The outside binding sites are most likely associated with the membranes and the inside one are due to binding to the nucleus. In addition, studies with ghost membranes indicate that these drug bind to the membranes of the ghost most probably in the hydrophobic regions.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE INSTITUTE: Since the carcinogenic and mutagenic properties of antitumor drugs are most likely associated with DNA binding, it is of great significance to understand the mechanism of actions of these drugs at a molecular level. It is hoped that such studies may lread to better understanding of the chemical properties that are responsible for the carcinogenic and antitumor properties of these agents.

TABLE-1: INTERACTION OF ANTITUMOR DRUGS
WITH MSL LABELED ERYTHROCYTE MEMBRANES[a]

	W/S ratio[b]	
Drug	$1x10^{-5}$M	$5x10^{-5}$M
Control	5.85	
Daunomycin	6.135 (4.8)	6.1 (4.8)
Adriamycin	6.37 (8.8)	6.54 (11.8)

Actinomycin D	6.45 (10.2)	6.58 (12.5)
AMSA	6.58 (12.5)	6.71 (14.7)
Diacridine (C_8)	6.17 (5.5)	5.21 (11)
cis-Pt Complex	6.58 (12.5)	6.58 (12.5)

[a] Contained 2.5 - 3.0 mg protein/ml.

[b] The values represent the ratio of weakly to strongly immobilized labels. The % increase or decrease is given in parenthesis.

TABLE 2: INTERACTION OF ANTITUMOR DRUGS WITH MSL LABELED MURINE MASTOCYTOMA P 815 CELLS[a]

Drug	W/S ratio[b] 1 x 10^{-5} M	5 x 10^{-5} M
Control	7.22	
Daunomycin	7.81 (8.2)	7.97 (10.4)
Adriamycin	7.71 (6.8)	7.63 (5.7)
Actinomycin-D	7.680 (6.4)	7.684 (6.4)
AMSA	7.94 (10)	8.366 (15.8)
Diacridine (C_8)	8.132 (12.6)	7.786 (7.8)
cis-Pt complex	8.333 (15.4)	8.476 (17.4)

[a] contained 1.1 x 10^8 cells/ml.

[b] The values represent the ratio of weakly to strongly immobilized labels. The % increase or decrease is given in parenthesis.

PUBLICATION

Sinha, B.K. and Chignell, C.F.: Interactions of Antitumor Drugs with Human Erythrocyte Ghost Membranes and Mastocytoma P 815: A Spin Label Study. *Biochem. Biophy. Res. Commun.* 86: 1051-1057, 1979.

Sinha, B.K. and Chignell, C.F.: The synthesis and use of spin-labeled analogs of Biotin in the study of avidin in *Methods in Enzymology* (D.B. McCormick and L.D. Wright eds) 62: 295-307, 1979.

Chignell, C.F. Spin labeling in pharmacology in *Spin Labeling II Theory and Applications* Academic Press, New York, 223-246, 1979.

Rakhit, A. and Chignell, C.F.: A spin label study of horseradish peroxidase. *Biochim. Biophys. Acta* in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 50054 Z01 ES ~~5054-01~~ LEB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
The Free Radical Formed Microsomal Incubations Containing CCl_4 and NADPH

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Kalyanaraman	Visiting Fellow	LEB	NIEHS
OTHERS:	R.P. Mason	Research Chemist	LEB	NIEHS
	E. Perez-Reyes	Biological Tech.	LEB	NIEHS
	C.F. Chignell	Chief	LEB	NIEHS
	C.R. Wolf	Visiting Associate	LP	NIEHS
	R.M. Philpot	Research Chemist	LP	NIEHS

COOPERATING UNITS (if any)

Laboratory of Pharmacology

LAB/BRANCH
Laboratory of Environmental Biophysics

SECTION
Molecular Biophysics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The hepatotoxicity of carbon tetrachloride is usually thought to be due to the enzymatic formation of the trichloromethyl radical. A variety of indirect, but not conclusive, evidence for the formation of $\cdot CCl_3$ exists: hydrogen abstraction by $\cdot CCl_3$ to form $CHCl_3$ and dimerization of $\cdot CCl_3$ to form C_2Cl_6. Hydrogen abstraction of a methylene hydrogen from polyunsaturated fatty acids by the trichloromethyl radical would be followed by oxygen addition and would result in lipid peroxidation. Carbon tetrachloride-induced lipid peroxidation has been extensively studied both in vitro and in vivo. Attempts to use electron spin resonance (ESR) spectroscopy to demonstrate directly the presence of the trichloromethyl radical in hepatic microsomal incubations or liver slices have been unsuccessful. Recently a free radical has been detected in microsomal incubations containing NADPH and CCl_4 or $CBrCl_3$ using the spin-trap phenyl-*t*-butyl nitrone (PBN). This free radical adduct was identified as the $\cdot CCl_3$ adduct of PBN. Our studies have shown, however, that a lipid dienyl radical, similar to that formed by the action of soybean lipoxygenase on linoleic acid, is the species being trapped.

PROJECT DESCRIPTION

METHODS EMPLOYED: Electron spin resonance (ESR) spectroscopy, in conjunction with spin-trapping agents, has been used to detect steady state levels of free radicals.

MAJOR FINDINGS AND PROPOSED COURSE: With the aid of the spin-trapping technique, Poyer, *et al.* have detected a free radical in microsomal incubations containing NADPH and CCl_4 or $CBrCl_3$ using the spin-trap phenyl-*t*-butyl nitrone (PBN). This free radical was identified as the trichloromethyl adduct of PBN based on the similarity of its ESR spectrum to that of the free radical formed by UV photolysis of a CCl_4 solution of PBN. Ingal *et al.* using the spin trap 2-methyl-2-nitrosopropane (MNP), have also trapped a free radical in microsomal incubations containing CCL_4 and NADPH, but in this case, the spectrum was not the same as that generated by the X-ray irradiation of a CCl_4 solution of MNP. The latter spectrum was claimed to be that of the MNP-trichloromethyl spin adduct based on an isotopic effect seen with ^{13}C-carbon tetrachloride. However, it should be noted that this spectrum of the MNP-trichloromethyl radical adduct is clearly different from earlier spectra in that hyperfine structure due to chlorine was not observed. Based on the differences between the microsomal and X-ray irradiation spectra, Ingall *et al.* concluded that the free radical trapped in the microsomal experiments was probably either $CCl_3\text{-}O_2^{\cdot}$ or a secondary lipid peroxyl radical rather than the $^{\cdot}CCl_3$ radical. Our spin-trapping investigations with PBN and MNP have demonstrated that these interpretations are in error, and that in both cases a lipid dienyl free radical, similar to that formed by the action of soybean lipoxygenase (linoleate: oxygen oxidoreductase, EC 1.13.11.12) on linoleic acid, is probably the species that is trapped.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND PROGRAM OF THE INSTITUTE: CCl_4 toxicity results from metabolic activation of this agent by the liver, which is the main site of CCl_4-induced pathological changes. This activation is thought to require the homolytic cleavage of one of the chlorine-carbon bonds of CCl_4, to form the trichloromethyl free radical. From this single unproven event all of the entire spectrum of pathological consequences of CCl_4 poisoning is thought to follow. The central importance of this proposed free radical metabolite to the hepatotoxicity of CCl_4 makes a demonstration of its existence in a biological system of considerable importance.

UNIVERSITY OF CALIFORNIA AT LOS ANGELES (UCLA)

TITLE: "Effects of 60 Hz Fields on the Mammalian Central Nervous System"

CONTRACTOR'S PROJECT DIRECTOR: W. Ross Adey, M.D.

PROJECT OFFICER (NIEHS): Donald I. McRee, Ph.D., Research Physicist
Laboratory of Environmental Biophysics

DATE CONTRACT INITIATED: October 1, 1975

CURRENT ANNUAL LEVEL: (Zero funding - extended into FY 1979 without additional funds)

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this research are to determine the effects of 60 Hz fields from high voltage transmission lines on the central nervous system of mammals.

METHODS EMPLOYED: Environmental field conditions similar to those found in the vicinity of high voltage transmission lines will be simulated. These field gradients will range from .1 to 1000 volts/meter. The effects of these fields on the biological rhythms in rats will be evaluated. The subjects were exposed continuously for 30 days except for short periods for maintenance and data collection intervals. The effects on the neuroendocrine mechanism in rats will be examined by using 17-hydroxysteriods, calcium, phosphorus, vanillylmandelic acid and other urinary indicators of biorhythmicity.

MAJOR FINDINGS AND PROPOSED COURSE: Exposures of rats to 60 Hz electric fields at 50, 500, and 1000 volts/meter (V/m) have been completed. Exposures at 500 and 1000 volts/meter and a complete sham exposure have been duplicated with an interval of approximately six months between these paired tests. The overall pattern of results showed no substantial effects associated with the 60 Hz field at any of the gradients tested. Two potential field-dependent effects, both in behavioral measures, were found: the relatively larger amount of activity recorded during the 0900-1000 period during the 1000 V/m condition, when compared with the other conditions, and the reduced amount and duration of late-night activity under the 1000 V/m condition when compared with the other conditions.

The strongest effect seen in the experiment was the reduction in activity counts/hour over the course of the experiment. This effect is field-gradient independent, but is strongly associated with the subject's size (when size is indexed by the subjects' weight, the correlation between activity counts and weight is -.85, accounting for 71% of the variability in the activity data).

The other potential field-dependent effect involved the apparent reduction of the late-night activity bout in the 1000 V/m rats, as compared to the control and 500 V/m rats. The "field on" effect suggested would be considerable interest, but a substantial amount of additional data would have to be analyzed before statistical analyses would be appropriate. The mechanisms by which a 1000 V/m 60 Hz electric field might change the size and duration of the late-night activity bout is unclear.

No field-dependent effect on increases in weight over the course of the experiment were observed. No significant difference in average urine volume per day per animal was measured for any of the field intensities. Electrolytes, Ca, Na, and K, and steroid levels in the collected urine, (as measured by atomic absorption spectroscopy) over the course of the experimental treatments showed no field-dependent effects. The gross characteristic of glandular tissue (adrenals and thyroid) showed no field-related effects. A detailed clinical picture of the blood that was taken at the completion of the experimental treatment did not show any significant changes.

NIEHS has completed its funding of this contract. Additional research relative to this project will be funded by the Department of Energy.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The electric power industry is planning to construct high voltage transmission lines with voltages up to 1200 kV. In the vicinity of the lines, high electric field gradients will be present. The health effects of these fields are presently unknown. This research will contribute to the determination of whether these electric fields do or do not constitute a health hazard. This project contributes to the mission of the Institute in evaluating the health effects of physical factors in the environment.

PUBLICATIONS

Adey, W.R.: Models of membranes of cerebral cells as substrates for information storage. BioSystems 8: 163-178, 1977.

Adey, W.R.: The sensorium and the modulation of cerebral states: tonic environmental influences on limbic and related systems. Ann. New York Acad. Sci., 290: 396-420, 1977.

Bawin, S.M., Sheppard, A. and Adey, W.R.: Mechanisms of weak electromagentic field coupling in brain tissue. In Taylor, L.S. and Cheung, A.Y. (Ed.): The Physical Basis of Electromagnetic Interactions with Biological Systems, University of Maryland. Office of Naval Research and Bureau of Radiological Health, Food and Drug Administration, pp. 75-89, 1977.

Bawin, S.M., Sheppard, A. and Adey, W.R.: Models of long-range order in cerebral macromolecules: effects of ELF, VHF and UHF fields on calcium binding. URSI International Symposium on the Biological Effects of Electromagnetics Waves, Airlie, Virginia, 1977.

Bawin, S.M., Sheppard, A. and Adey, W.R.: Possible mechanisms of weak electromagnetic field coupling in brain tissue. *Bioelectrochemistry and Bioenergetics*, 1978.

UNIVERSITY OF UTAH

TITLE: "Effects of Microwave Radiation on the Nervous System"

CONTRACTOR'S PROJECT DIRECTOR: Om P. Gandhi, Ph.D.

PROJECT OFFICER (NIEHS): Donald I. McRee, Ph.D., Research Physicist
Laboratory of Environmental Biophysics

DATE CONTRACT INITIATED: June 28, 1976

CURRENT ANNUAL LEVEL: (Zero No cost extension to December 28, 1978)

PROJECT DESCRIPTION

OBJECTIVE: The objective of this project is to determine the effects of long-term, low-level exposure to 915 and 2450 MHz microwave radiation on the central nervous system and behavior.

METHODS EMPLOYED: Long-Evans male rats will be exposed to a CW plane wave microwave field at frequencies of 915 and 2450 MHz and power density of 5 mW/cm^2. The animals will be exposed for 8 hours a day, 5 days a week for 16 weeks. An eight week recovery period will follow the exposure period. The following measurements will be made:

1. Biochemistry - Biochemical analysis of the cholinesterase activity of the blood, the SH group in the blood, and ketosteriods in the urine will be made.
2. Behavior - Rodent activity will be measured using a rodent activity wheel. Water and food intake will be measured. Rats will be trained to lever press for food reward on a FR-DRL multiple schedule. Total response and reinforcement for each schedule component as well as response during time out between each component will be recorded. Interesponse times on each schedule component will be recorded.
3. EEG - EEG will be recorded biweekly using epidural removable stainless steel electrodes. These recordings will be made while the microwave radiation is turned off.

MAJOR FINDINGS AND PROPOSED COURSE: Fifteen male Long-Evans adult rats were exposed for sixteen weeks to 2450-MHz CW microwaves at an average power density of 5 mW/cm^2. The resulting dose rate was 1.23 ($\pm$0.25 SEM) mW/g. The animals were exposed eight hours each day for a total of 640 hours in a monopole-above-ground radiation chamber while housed in Plexiglas holding cages. Daily measures of body mass and of food and water intake indicated no effect of microwave irradiation. Biweekly stabilimetric activity tests indicated a significant depression of locomotor activity by the fifteen microwave-exposed rats as compared with fifteen sham-exposed animals, when measured immediately after exposure. Measures of activity based on revolutions of running wheel, which were obtained during twelve-hour periods between each eight-hour exposure, showed no significant effect of microwaves. Blood sampled at 2, 6, 10 and 14 weeks of exposure indicated slight alterations of sulfhydryl groups, and of red and white blood-cell counts. Measures of 17-ketosteroid levels in urine at weeks 1, 5, 9 and 12 of exposure and mass of adrenals, heart, and liver at the end of the sixteen-week period of exposure revealed no indications of stress. The project has been completed and a final report will be received by the end of FY 1980.

SIGNFICIANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The biological effects of long-term, low-level microwave exposure on the central nervous system and behavior is not known. Presently, only a small amount of research is being performed to evaluate the health hazards of this type of radiation. This research will contribute to the determination of whether these fields do or do not constitute a health hazard. This project directly applies to the mission of the Institute in evaluating the health effects of physical factors in the environment.

PUBLICATION

D'Andrea, J.A., Gandhi, O.P., Lords, J.L., Durney, C.H., Johnson, C.C. and Astle, L.: Physiological effects of chronic exposure to 2450-MHz microwaves. J. of Microwave Power (In Press).

UNIVERSITY OF MIAMI

TITLE: "Protracted Noise Exposure and Cardiovascular Function"

CONTRACTOR'S PROJECT DIRECTOR: Ernest A. Peterson, Ph.D.

PROJECT OFFICER (NIEHS): Teruzo Konishi, M.D., Ph.D., Visiting Scientist
Laboratory of Environmental Biophysics

DATE CONTRACT INITIATED: January 1977

CURRENT ANNUAL LEVEL: $40,000

PROJECT DESCRIPTION

OBJECTIVES: Because of the serious and pervasive nature of cardiovascular diseases, including hypertension, the relationship between noise and cardiovascular function has received particular attention. The objectives of this contract are to specify (1) the extent to which protracted noise exposure produces cardiovascular adjustments, (2) the dynamics of those adjustments, and (3) the nature and mechanisms of adaptation to noise. Semi-restrained rhesus monkeys are used for the work.

METHODS EMPLOYED: Rhesus monkeys will be exposed for a period of up to one year to noise conditions resembling those presently and potentially existing in the community and work place. Their cardiovascular responses will be compared to those of a group of rhesus monkeys maintained in relatively quiet conditions during the same period. Evidence for acoustic injuries and other organic pathology will be sought. During the course of the study, the general health condition will be monitored by various clinical tests in addition to appropriate physiological and biochemical tests relevant to cardiovascular function.

MAJOR FINDINGS AND PROPOSED COURSE: Two experimental monkeys were successfully exposed to term (8 months), however loss of 2 consecutive control monkeys due to catheter failure seriously hinders data interpretation at this point. Data is currently being collected from a third control. The existence of data in the literature regarding the blood pressures of unrestrained rhesus monkeys provides an indirect reference base. The blood pressures of the two experimental monkeys after six months of chair restraint but no noise exposure were at the 50th percentile with respect to the indirect reference base but the diastolic pressures had risen to the 99th percentile after six months of noise exposure. The results appear to implicate stroke volume changes rather than increase in peripheral resistance as the source of the systolic rise, since pulse pressure has not decreased but risen. In the second phase of the experiment, the investigator has planned to measure stroke volume directly by placing a flow probe on the aortic arch. The investigator has been encouraged to submit a protocol for the measurement of other physiological (dpdT, epicardial EKG, and cardiac output) and biochemical (blood chemistry profile including SMA 40 and plasma catecholamine and cortisol level) parameters in the second phase of the project.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The work will provide scientific information which is vital to futher refine our concepts of how long-term exposure to contemporary noise levels affects the cardiovascular system in humans.

UNIVERSITY OF WASHINGTON

TITLE: "Effects of Microwave Radiation on the Nervous System"

CONTRACTOR'S PROJECT DIRECTORS: Richard H. Lovely, Ph.D.
Arthur W. Guy, Ph.D.

PROJECT OFFICER (NIEHS): Donald I. McRee, Ph.D., Research Physicist
Laboratory of Environmental Biophysics

DATE INITIATED: June 20, 1976

CURRENT FUNDING LEVEL: $30,000

PROJECT DESCRIPTION

OBJECTIVE: The objective of this project is to determine the effects of long-term, low-level, exposure to 918 and 2450 MHz microwave radiation on the central nervous system and behavior.

METHODS EMPLOYED: Long-Evans male rats, 80-90 days of age will be exposed to 915 and 2450 MHz circularly polarized microwave radiation at a power density level such that the specified absorption rate will be equivalent to that produced in a 5 mW/cm^2 plane wave field. The exposure will be 8 hours a day, 7 days a week for 4 months. Two month post-irradiation evaluations will be performed. Rats will also be exposed to 2450 MHz plane wave microwave field at a power density of 500 μW/cm^2 using the same schedule as that stated above. The following indicators of biological effects will be made:

1. Biochemistry - Serum content for sodium, calcium, phosphorus, PTH, SH groups and cholinesterase activity of the blood and 17-ketosteriods in urine will be measured.
2. Behavior - Animals will be evaluated for free-operant and free-repertoire behavior. Animals will be observed in the irradiation chambers and their specific orientation to the field, sleep, grooming, sniffing, rearing, drinking, eating, etc., will be recorded.
3. Electroencephalography - EEG recordings will be made on the animals following the four month irradiation period.

In addition to the above endpoints, some behavioral teratology, data will be obtained.

MAJOR FINDINGS AND PROPOSED COURSE: Eight male rats were exposed for 16 weeks to 2450 MHz circularly polarized microwaves at an averaged power density of 5 mW/cm^2. The rats were exposed for ten hours every night for a total of approximately 1100 hours while 8 other rats were sham-exposed for the same time periods. Daily measurements of body mass, and of intake of food and of water, both in home cage and exposure apparatus, revealed statistically significant decrements in the exposed rats' daily food and water intake, although body mass failed to differentiate groups. Monthly assessments of blood chemistry parameters also failed to differentiate groups in a consistent manner as did post-exposure determinations of footpad shock threshold, open field behavior, and the acquisition of a shuttlebox conditioned avoidance response. When the present findings at 2450 MHz (e.g., reduction in food intake) are compared to the 918 MHz dose-response function in terms of incident energy, the effects common to both 918 MHz and 2450 MHz chronic exposure are frequency dependent. However, when the effects are plotted as a function of SAR (W/Kg), the effects obtained here can be easily predicted from the 918 MHz dose-response curve.

Eight pregnant rats were exposed to 500 $\mu W/cm^2$, 2450 MHz, circularly polarized guided waves, for twenty hours a day throughout the first nineteen days of gestation. There were no distinct physical characteristics which differentiated exposed from sham- and non-exposed litters at birth. However, during the following two weeks, a series of phenomena emerged which implied some type of *in-utero* microwave effects. There were seven times as many neonatal deaths in the exposed litters than in the controls. Body weights on day 7 of life were significantly lower in exposed males and females relative to control offspring. The pups exposed prenatally lagged behind by 36 hours in initiation of eye openings. In addition, subsets of the treated pups were fostered (F-pups) to naive mothers after being culled from the above mentioned litters, which left the remaining pups with the natural mothers (N-pups). The F-pups were then exposed or sham-exposed postnatally, two hours daily from day 4-11 of life. The prenatally exposed female F-pups demonstrated a significant inability to retain body heat when sham-exposed postnatally. All prenatally exposed females demonstrated an accelerated growth rate between 49-84 days of age which led to subsequent maintenance of a 10% higher body weight throughout adulthood. As adults, the exposed female N-pups that were tested in a conditioned avoidance task exhibited a facilitation in the acquisition phase, while similarly treated male N-pups did not. On the other hand, prenatally exposed male F-pups when subjected to cold stress (5°C) as adults, were unable to maintain normal core temperatures relative to controls, while female pups showed no such difficulty. It was concluded that developmental neurobiologic changes had occurred as a result of *in-utero* exposures to microwaves. This project has been completed. Research to further investigate these biological changes are being planned through additional contract support.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The Institute has, as part of its mission, the responsibility for studying the biological effects of physical factors in the environment. The potential health effects of low-level, long-term microwave exposure are not now known. With the increase of microwave radiation in the environment, it is important to determine the effects of microwave exposure. The most often reported effect in the literature is on the central nervous system and behavior. It is important that the Institute study long-term, low-level, effects since very little research at these conditions is presently being done in the United States.

PUBLICATION

Johnson, R.B., Mizumore, S., and Lovely, R.H.: Adult behavioral deficit in rats exposed prenatally to 918 MHz microwaves. Proceedings 17th Hanford Biology Symposium. (In press).

RESEARCH TRIANGLE INSTITUTE

TITLE: Investigation to Determine the Peripheral and Central Receptor Mediating Effects of Microwave Radiation on Brain Activity

CONTRACTOR'S PROJECT DIRECTOR: Blake S. Wilson, Senior Research Engineer

PROJECT OFFICER (NIEHS): Donald I. McRee, Ph.D., Research Physicist
Laboratory of Environmental Biophysics

DATE CONTRACT INITIATED: June 1, 1979

CURRENT FUNDING LEVEL: $40,000

PROJECT DESCRIPTION

OBJECTIVES: The objectives of the research is to identify the biological structures that transduce microwave radiation into auditory activity and to investigate possible effects of microwave radiation on the metabolic activity of brain structures outside the auditory system.

METHODS EMPLOYED: Responses of single fibers in the auditory nerve of cats will be recorded using glass micropipette electrodes while the head is being exposed to either continuous wave or pulsed microwave radiation. Post stimulus time histograms of the neural discharge data will provide latency information which can be used to determine the site of interaction. To investigate the interaction of microwaves with various brain centers, patterns of $[^{14}C]$2-deoxy-D-glucose ($[^{14}C]$2 DG) uptake in brain centers will be measured. Brain metabolism can then be measured by obtaining autoradiographs of specific loci of the brain.

MAJOR FINDINGS AND PROPOSED COURSE: Patterns of evoked activity in auditory nerve fibers to pulses of microwave radiation and to acoustic clicks have confirmed the hypothesis of a transduction of microwave energy into mechanical disturbances peripheral to or at the baselar membrane. In addition some evidence for a direct effect of microwaves at the sensory hair cells of the cochlea has been obtained. Patterns of $[^{14}C]$2 DG utilization at the levels of the inferior colliculus and medial geniculate body of rats with one middle ear oblated have shown that exposure to pulsed microwave radiation can elicit a metabolic response in the auditory system by some mechanism other than conduction of sound through the middle ear.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: At the present time the mechanisms of microwave interaction with the brain are unknown. This study should provide information on the nature of the receptors that are responsible for mediating the effects of microwave radiation on brain activity.

NEW YORK UNIVERSITY

TITLE: "Photodegradation of Absorbed Polycyclic Arenes"

PRINCIPAL INVESTIGATOR: Joan M. Daisey, Ph.D., Institute of Environmental Medicine, NYU Medical Center

PROJECT OFFICER (NIEHS): Colin F. Chignell, Ph.D., Chief, Laboratory of Environmental Biophysics

DATE GRANT INITIATED: December, 1978

CURRENT ANNUAL LEVEL: $26,000

GRANT DESCRIPTION

OBJECTIVES: Although it is to be expected that polycyclic aromatic hydrocarbons (PAH) absorbed onto solids e.g. fly ash, soil praticles will undergo photochemical degradation little is known about either the mechanism of this reaction or the products that are formed. The purpose of this investigation is to provide more adequate information for the assessment of environmental and occupational health hazards of airborne PAH and reactive intermediates formed during the photodegradation of absorbed polynuclear aromatic hydrocarbons.

METHODS EMPLOYED: A fluidized bed photochemical areactor will be developed and evaluated for the study of particulate-absorbed PAH and PAH-epoxides. The stability of some reactive epoxide intermediates which may be formed during the photodegradation of absorbed PAH (in aerosols) will be determined under various conditions of light, temperature and humidity. The rate of photodegradation and chemical half-lives of pyrene, benz(a)anthracene and benzo(a)pyrene absorbed onto several substrates will be determined. Identification of the products formed during photodegradation will be made with particular emphasis on arene epoxide derivatives.

MAJOR FINDINGS AND PROPOSED COURSE: Preliminary experiments have been carried out to select appropriate substrates for this study. Initial work has shown that glass beads are suitable, although other substrates including carbonaceous materials and silica are still being evaluated. Several coating processes are also being examined to determine which will provide a suitable procedure for absorbing the PAH onto the substrates. The fluidized bed photoreactor has been designed and is in the process of being constructed. A xenon-arc lamp will be used for the exposure studies since it provides a wide spectrum of wavelengths similar to those found in sunlight. The PAH photodegradation products will be isolated from the particles by solvent extraction. Identification of the products will be achieved using conventional chemical analysis procedures e.g. as chromatography mass spectrometry, high performance liquid chromatography, absorption and fluorescence spectroscopy and NMR.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Effluents from such processes as oil shale conversion and coal gasification contain high concentrations of PAH. Once released into the environment these agents may be absorbed into soil particles and other substrates. Since photochemical degradation in the presence of oxygen could give rise to intermediates that are more chemically reactive than the parent compounds it is of importance to isolate and identify such compounds.

LABORATORY OF ENVIRONMENTAL TOXICOLOGY

LABORATORY OF ENVIRONMENTAL TOXICOLOGY
Summary Statement

The Laboratory of Environmental Toxicology (LET) has recently been restructured to focus more directly on problems involving reproductive, developmental, and endocrinological toxicity. Two workgroups, formerly Biochemical Toxicology and Developmental Toxicology, have been transferred to form the nucleus for a new laboratory, Laboratory of Organ Function and Toxicology (LOFT). A Chief for this organization is being recruited; the laboratory title accurately describes its area of responsiblity. A summary of LOFT research activities follows this summary for LET.

Reproductive and developmental toxicology has generally lagged behind fields such as cellular and molecular biology with regard to laboratory approaches. The detection of environmental agents which affect reproduction or product birth defects is unsure and the underlying biological mechanisms which account for these major health problems are unclear. Thus, the Laboratory of Environmental Toxicology bridges the gap between the most molecular aspects of reproductive biology and endocrinology and the more applied problems associated with the detection of hazardous chemicals, the extrapolation of laboratory data to man, and the estimation of human risk.

Human health problems in this general area are abundant. About 3.5 million children are born alive in the United States each year. It can be reasonably estimated that 240,500 (7%) of these will have congenital malformations; 42,000 (1.2%) will die before one month of age, and nearly 14,000 (0.4%) more children will die before their first birthday. There is also a high incidence of spontaneous abortions, and a large number of premature and low birth weight babies. Perhaps even more important from the standpoint of environmental exposures, are the more subtle postnatal effects which occur later in life. Gestational exposure to diethylstilbestrol can induce reproductive tract abnormalities in both female and male offspring and cancer in females during their teenage years. There is also an apparent increase in human infertility. Dibromochloropropane (DBCP), an agricultural nematocide, has been related to male infertility following occupational exposure. Many of these problem areas are described in the Report of the Second Task Force for Research Planning in Environmental Health Sciences, Human Health and the Environment: Some Research Needs. Our research program is directly responsive to the Task Force's recommendations.

The Laboratory directs its research efforts to three major areas at the present time: (1) male and female reproductive processes; (2) teratogenesis; and (3) postnatal effects of gestational chemical exposure. Studies are conducted focusing on the entire biological spectrum from molecular aspects to whole animals. Organizationally, the Laboratory consists of five administrative units or workgroups: Molecular Embryology, Experimental Teratogenesis, Transplacental Toxicology, Developmental Pathology, and Germ Cell Toxicity. However, extensive collaboration exists between these groups; thus, the current research accomplishments for the Laboratory are listed below as integrated programs at various levels.

I. Molecular Level

A. Hormone-related gene action in the male and female genital tract

- o Hormone regulation of prostate and seminal vesicle protein synthesis.
- o Gene cloning for androgen dependent rat prostate proteins.
- o DNA/RNA characterization of androgen dependent rat seminal vesicle proteins.
- o Protein mapping of the developing genital tract of the fetal and neonatal mouse.

B. Biochemical basis of estrogen action

- o Characterization of nuclear events involved in estrogen action in the mouse uterus.
- o Structural requirements for estrogen activity with emphasis on diethylstilbestrol and its metabolites.

II. Ultrastructural/Cellular Level

A. Ultrastructural changes as predictors of functional abnormalities

- o Correlation of scanning and transmission electron microscopic observations as precedents of biochemical, histologic or functional changes in the male and female mouse genital tract.

B. Toxication/Detoxication of environmental chemicals by target tissues related to reproduction

- o Polycyclic aromatic hydrocarbon metabolism by rodent testes.

- o Development of a coupled system of microsomal enzymes and cultured rodent fetuses to determine the role of metabolism in teratology.
- o Characterization of uterine bioactivation of diethylstilbestrol and the elucidation of metabolic pathways of differing biological activities.

C. Toxicology of early development

- o Interspecies in vitro fertilization as an indicator of reproductive capacity.
- o In vitro development of fertilized eggs through early embryonic stages to determine developmental integrity.

III. Tissue/Organ Level

A. Cultured fetuses

- o Establishment and biochemical/physiological/morphological characterization of an in vitro system to grow and maintain whole rat fetuses during critical periods of organogenesis.

B. Isolated development of fetal organs

- o Establishment and biochemical/physiological/morphological characterization of an in vitro system to grow and maintain fetal mouse genital tracts and gonads during the period of estrogen sensitivity.
- o Morphologic and functional characterization of heterologous cultures of testes and Müllerian ducts derived from DES-exposed and unexposed fetal mice.
- o Morphological characterization of long term fetal tissue grafts.
- o In vitro brain cell aggregation as a predictor of teratogenesis.

IV. Whole Animal Studies

A. Toxicology

- o The teratogenicity of anticonvulsant agents and structurally related chemicals.

- Characterization of reproductive tract function (including fertility and carcinogenicity) and immune capacity in male and female mice exposed *in utero* to diethylstilbestrol.
- Postnatal effects of gestational exposure to methyl butyl ketone, ethrane or ethchlorovinyl (Placidyl).
- Transplacental toxicity of selected environmental chemicals in rats: zeranol, several PCB and PBB isomers, and polycyclic hydrocarbons.
- Effects of DBCP on male reproductive function in mice.
- Effects of kepone on male reproductive function in mice.

B. Data extrapolation to man and risk estimation

- Diethylstilbestrol-exposed mouse offspring as a model for similarly exposed humans.
- Quantitation of chemical teratogenicity relative to maternal toxicity as a possible model for predicting relative human risk.
- Testicular compartment model of pharmacokinetic and adaptive processes which aids interspecies comparisons.

Summaries of these projects are presented below; details of the work appear in the individual annual reports.

I. Molecular Level

A. Hormone-related gene action in the male and female genital tract

Hormone regulation of prostate and seminal vesicles protein synthesis. Protein synthesis patterns analyzed by two dimensional (2D) gel electrophoresis in castrate and testosterone stimulated prostate and seminal vesicle indicate that a major group of secretory proteins in both organs is under androgen control. Both organs have a high concentration of poly(A^+)-mRNA which code in a wheat germ translation system for polypeptides. The two major poly(A^+)-RNA's from prostate (labeled β and γ) code for the subunits of the major secretory product referred to as prostate binding protein. These two prostate poly(A^+)-mRNA make up 30-40% of the total poly(A^+) of the prostate. Likewise, rat seminal vesicle has two major poly(A^+)-mRNA's (40%) which code for two major seminal vesicle proteins which are androgen responsive.

Gene cloning for androgen dependent rat prostate proteins. Double-stranded complementary DNA (ds cDNA β and γ) to androgen dependent prostate β and γ poly(A$^+$)-RNA was prepared using reverse transcriptase. The synthetic gene ds cDNA β was separated from the ds cDNA γ by preparative agarose gel electrophoresis. The relative pure ds cDNA to both γ and β were then restricted with a variety of restriction enzymes and preliminary maps generated for the two synthetic prostate genes. A large batch of ds cDNA β and γ was synthesized (~2 μg), treated with S1 nuclease, tailed with terminal deoxynucleotide transferase, and cloned in the plasmid pBR322. Clones were identified which had a high probability of containing either β or γ ds cDNA inserts by colony filter hybridization. Cultures of these clones were grown; the plasmids isolated are presently under investigation using a variety of restriction enzymes. The prostate β and γ clones will be further characterized in the future, and probes for specific regions of these genes developed. With characterized probes for these androgen regulated genes, molecular and environmental factors affecting these specific genes can be studied.

DNA/RNA characterization of androgen dependent rat seminal vesicle proteins.
Double-stranded complementary DNA for two major seminal vesicle poly(A$^+$)-mRNA's was prepared (ds cDNA to mRNA$_{sv}$). The two seminal vesicle synthetic genes were purified and isolated and restriction maps generated. It appeared as if the structural parts of these two seminal vesicle genes have considerable sequence homology. A 11S poly(A$^+$)-mRNA comprised 40% of the total poly(A$^+$)-mRNA in the seminal vesicle. This 11S poly(A$^+$)-mRNA appears as two major bands in agarose gel electrophoresis under denaturing conditions. The size of the two poly(A$^+$)-mRNA bands are 650 NT (mRNA$_{sv1}$) and 580 NT (mRNA$_{sv2}$). Poly(A$^+$)-mRNAs enriched for mRNA$_{sv1}$ code in a wheat germ translation system for a polypeptide of 18,000 daltons, similar in molecular weight but slightly larger than the seminal vesicle secreted protein IV. Likewise, the mRNA$_{sv2}$ appears to code for a polypeptide of 14,000 daltons, again similar and slightly larger than the secreted protein V. The seminal vesicle ds cDNA to mRNA$_{sv1}$ and mRNA$_{sv2}$ will be cloned and probes developed for these genes. With characterized probes for these androgen regulated genes, a detailed analysis of their structure and function in the chromosomal DNA becomes feasible.

Protein-mapping of the developing genital tract of the fetal and neonatal mouse.
The protein-mapping technique of O'Farrell is being used to study the synthesis of total protein in embryonic and postnatal mouse genital tracts. Normal differentiation in Müllerian and Wolffian ducts from mouse fetuses beginning with day 14 through birth is being followed by ^{35}S-methionine incorporation into protein. High resolution maps of immature and mature mouse uteri are also being developed to establish end points of differentiation. There are approximately 400 protein spots detectable in each pattern developed thus far. Some 5% of the detectable proteins are either sex or developmental stage specific. The effects of prenatal and postnatal diethylstilbestrol exposure on fetal and immature uteri protein synthetic patterns will be assessed.

B. Biochemical basis of estrogen action

Characterization of nuclear events involved in estrogen action in the mouse uterus. Characterization of uterine nuclear estrogen receptors in the mouse suggests two forms of the receptor, since the mouse uterus possesses a second translocation of hormone receptor complex to the nucleus after exposure to hormone. Compounds with poor estrogenic potency lack the ability to elicit this second nuclear peak. The role of this event in estrogen action in the mouse reproductive tract, with particular regard to the actions of hormonally active environmental chemicals, is being studied. Receptor resynthesis, RNA polymerase activities, DNA polymerase activities and glucose oxidation/-utilization are also being investigated.

Structural requirements for estrogen activity with emphasis on diethylstilbestrol and its metabolites. In order to determine whether the metabolism of DES resulted in biologically active or inactive metabolites, certain DES metabolites and analogs were tested for estrogenic activity using both an *in vivo* bioassay and an *in vitro* receptor binding assay. Results of these studies showed good correlation between the biochemical and bioassay data. Compounds such as DES-epoxide or catechol-DES were associated with reasonable receptor binding and biological activity; while certain metabolites, e.g. β-dienestrol or ω-hydroxy dienestrol, showed weak receptor interactions and poor estrogenicity. This indicates that the metabolism of DES does not result in complete inactivation. The exception to these results was indanyl-DES, a DES analog and possible metabolite, which shows receptor binding comparable to DES, but was 20-25 times less biologically active. These studies have suggested a mechanism of altered/differential clearance of these analog receptor complexes from the target cell nucleus.

II. Ultrastructural/Cellular Level

A. Ultrastructural changes as predictors of functional abnormalities

Correlation of scanning and transmission electron microscopic observations as precedents of biochemical, histologic or functional changes in the male and female mouse genital tract. Studies have demonstrated that scanning electron microscopy (SEM) provides a tool for the detection of early neoplastic changes. The surface ultrastructural features of the lumen of the female mouse genital tract was evaluated during the estrous cycle and during development in normal CD-1 mice. The hormone-dependence of fine structural features of the cell surface was demonstrated in ovariectomized, hormone-treated females where various characteristics of intact animals were experimentally induced. Subsequent studies on DES-treated mice indicate that cell surface features are directly related to abnormal cell differentiation. Changes in the cell surface are correlated with alterations in the histological features of DES-exposed offspring. Histochemical and high resolution electron microscopic studies also play an integral role in the evaluation of the mechanism of DES-toxicity. Enzyme histochemical studies are ongoing to determine the precise location of the metabolic enzymes, peroxidase, which may activate DES to a toxic intermediate.

B. Toxication/Detoxication of environmental chemicals by target tissues related to reproduction

Polycyclic hydrocarbon metabolism by rodent testes. Polycyclic hydrocarbon activating and deactivating enzyme systems have been studied in the rodent testes. Benzo(a)pyrene hydroxylase (AHH), epoxide hydrase (EH), glutathione S-transferase (GSH-ST), and cytochrome P-450 were determined in both the seminiferous tubule and interstitial cellular compartments. Similar studies were done with prostatic tissue. Tetrachlorodibenzo-p-dioxin (TCDD) induced AHH and EH in both the testis and prostate gland; a surprising 150 fold increase in AHH was noted in the prostate gland. AHH, EH, and P-450 were each increased in hypophysectomized male rats following reatment with luteinizine hormone (LH) while follicle stimulating hormone (FSH) or testosterone had no effect. These results suggest that LH-associated induction of testicular AHH, EH, and P-450 content is associated with the interstitial cell compartment. Since toxic effects of arene oxides on male germ cells are affected by various pharmacokinetic factors not represented in cell-free systems, the isolated perfused testis (IPT) was utilized to better understand the testicular metabolism of chemicals. The IPT was perfused with radio-labelled benzo-(a)pyrene and metabolites determined using high pressure liquid chromatography. Metabolites in the perfusate differed from those detected in testicular tissue, and testicular metabolism contrasted with that noted previously for the liver. The role of testicular activation of chemicals is important to the understanding of reproductive and genetic effects of chemicals on male germ cells.

Development of a coupled system of microsomal enzymes and cultured rodent fetuses to determine the role of metabolism in teratology. Many toxic chemicals and most mutagens and carcinogens require metabolic activation of the substrate to an active form. Microsomal biotransformation enzymes have recently been coupled to embryos grown in culture in a manner analogous to the Ames test where an S-9 activating system is in contact with cultured Salmonella bacterial strains. It has been demonstrated that cyclophosphamide, an anticancer agent which requires metabolic activation, has no adverse developmental effects on cultured embryos unless the microsomal enzymes are present. Enzyme and cofactor requirements are being optimized for embryonic growth and enzymatic activity. Thus, a mammalian test system has been developed which might quickly predict environmental chemicals which are either direct or indirect acting teratogens.

Characterization of uterine bioactivation of diethylstilbestrol and the elucidation of metabolic pathways of differing biological activities. It has been demonstrated that peroxidase, an enzyme inducible in estrogen target tissues, is able to metabolize DES to its major metabolite, β-dienestrol. Bioactivation of DES was determined by the non-extractable binding of radioactivity to DNA and protein after incubation of ^{14}C-DES with several activating systems including one derived from a target tissue, the mouse uterus. This peroxidase activating system was also studied in the hamster kidney, a non-genital target tissue for DES. The estrogenic activities of a series of

DES metabolites and analogs were determined. Results of studies suggest that DES metabolism follows alternative pathways resulting in either metabolites which retain estrogenicity or which lack activity. Determination of the biological significance of potentially activated metabolites of DES should aid in generalizations to other classes of estrogenic environmental chemicals.

C. Toxicology of early development

Interspecies in vitro fertilization as an indicator of reproductive capacity. Heterologous (human sperm/hamster egg) in vitro fertilization was used to assess fertilizing capacity of human males. These studies identified certain individuals as subfertile who have routinely determined normal sperm number, motility and morphology. This approach demonstrated that the usual parameters for semen analyses are unsure predictors of male subfertility; only the most extreme semen abnormalities reliably predict human subfertility. Thus, the in vitro penetration of laboratory animal ova by human sperm appears to be an improved technique to assess human fertilization potential. The epidemiologic application of this noninvasive, fast and relatively inexpensive technique is being explored.

In vitro development of fertilized eggs through early embryonic stages to determine developmental integrity. LET's available methodologies and the public interest in "test-tube" babies stimulated the linking of our in vitro fertilization and embryo culturing techniques. A "test-tube" baby is actually a product of in vitro fertilization, embryo culturing and transfer to a female recipient. Thus, we can use our established procedures for mice to determine if any developmental abnormalities or delayed postnatal effects such as behavioral changes are associated with the procedures.

III. Tissue/Organ Level

A. Cultured fetuses

Establishment and biochemical/physiological/morphological characterization of an in vitro system to grow and maintain whole rat fetuses during critical periods of organogenesis. To aid in the laboratory assessment of teratogens and the understanding of the molecular mechanisms underlying teratogenesis, an in vitro culture system for rat embryos has been established. Rat conceptuses of pregnancy day 10 can be grown continuously for 96 hrs with extensive differentiation of major organs. Utilizing this embryo culture, nutritional and hormonal requirements for embryonic development are being studied, and the system's predictiveness for chemical teratogens is being tested. Extensive development of major organs occurs which is comparable to in vivo differentiation during the same period; organogenesis is highly sensitive to direct acting alkylating agents such as tetraethylene melamine (TEM). A microsomal enzyme activation system has been coupled to the embryo culture system which allows the detection of indirect acting teratogens such as cyclophosphamide.

B. Isolated development of fetal organs

Establishment and biochemical/physiological/morphological characterization of an in vitro system to grow and maintain fetal mouse genital tracts and gonads during the period of estrogen sensitivity. The morphologic and functional characterization of heterologous cultures of testes and Müllerian ducts derived from DES-exposed and unexposed fetal mice has been established. Explants of fetal mouse gonads and genital tracts maintained in organ culture have been used to determine protein maps of developing tissues derived from DES exposed and unexposed animals. The biochemical studies are correlated with hormone response studies and morphological observations.

Morphologic and functional characterization of heterologous cultures of testes and Müllerian ducts derived from DES-exposed and unexposed fetal mice. A heterologous organ culture system including DES exposed or unexposed testes or Müllerian ducts has been used to determine the mechanism of Müllerian duct persistence and hyperplasia in DES treated males. Results suggest that the primary site of action is on the duct system rather than through a failure of the testis to synthesize or release Müllerian Inhibiting Hormone.

Morphological characterization of long term fetal tissue grafts. To overcome difficulties in long term (greater than 2 months) maintenance of fetal tissues in vitro, cultured fetal gonads or genital tracts are carried as grafts in appropriately manipulated hosts. Under these conditions, fetal ovaries develop into functional gonads when grafted in kidney capsules.

In vitro brain cell aggregation as a predictor of teratogenesis. In search of a simplified in vitro test system to predict both physical and chemical teratogens, the effects of total body maternal X-irradiation on cell aggregation patterns of enzymatically isolated fetal brain cells were studied. Maternal whole body X-irradiation on day 14 of gestation results in multiple birth defects including microcephaly and/or hydrocephalus. Thus, pregnant Sprague-Dawley rats were exposed on day 14 of gestation to a single dose of 24, 50, 100 or 200 R. In vitro brain cell aggregation in control groups was associated with development of membrane filopodia and a subsequent complex interaction of membrane filopodia between the cells and cell aggregates. In contrast, X-irradiation (>50 R) prevented the development of membrane filopodia with coincident inhibition of interactions between aggregates. Transmission electron microscopy also demonstrated treatment effects. These results indicate that perturbation of morphogenetic fetal brain cell interactions during differentiation are associated with malformations. Preliminary results suggest that membrane factors which influence cell aggregation might be a sensitive early target for teratogens. Ongoing studies will determine if changes in cell aggregation can be induced by chemical teratogens.

IV. Whole Animal Studies

A. Toxicology

The teratogenicity of anticonvulsant agents and structurally related chemicals. The teratogenicity of structurally related (cyclic imides) antiepileptic drugs was studied. Trimethadione, dimethadione, and paramethadione are derivatives of oxazolidinedione; diphenylhydantoin, ethotoin and mesantoin are hydantoins; and phensuximide, methsuximide and ehtosuximide belong to the succinimide class of compounds. All the drugs and each of the basic chemicals produced embryotoxic and teratogenic effects. The malformation profile observed in the CD-1 mouse were similar to those described in humans following in utero exposure during anticonvulsant therapy. Comparative computer analysis of the dose-related increase in the incidence of malformations and adult lethality for each of the compounds indicated that the relative teratogenicity of the oxazolidinedione class of anticonvulsants was significantly greater than that of hydantoins and succinimides. A common mechanism of action associated with the imide structure is suggested by the fact that each of these drugs share a common moiety and all are embryotoxic and teratogenic.

Characterization of reproductive tract function (including fertility and carcinogenicity) and immune capacity in male and female mice exposed in utero to diethylstilbestrol. Prenatal exposure to DES in mice results in a dose-related decrease in fertility and genital tract abnormalities in the offspring of both sexes; in females, tumors of the vagina, cervix, uterus and ovary were found. Immune function in both sexes was altered although not uniformly for males and females. These studies should be pertinent to the development of an animal model for similar human exposures.

Postnatal effects of gestational exposure to methyl butyl ketone, ethrane or ethchlorovinyl (Placidyl). The postnatal toxic effects of 2-hexanone, ethrane, and ethchlorvynol following gestational exposure were studied at five postnatal milestones: birth, weaning, puberty, adulthood, and old age. 2-Hexanone (methyl n-butyl ketone) was selected because of associated human neuropathies; ethrane is a halogenated anesthetic agent; and ethchlorvynol (Placidyl) is a sedative containing a vinyl chloride moiety in its structure. Effects observed included temporal difference in developmental indicators, changes in microsomal mixed function oxidase activity, altered blood and serum parameters, and some changes in behavioral test performances. All of these effects were considered minor; there were no histopathology nor any indication of carcinogenicity observed at the end of lifetime studies for any chemical studied. The fact that the human population now has a lifetime exposure to an increasingly large number of chemicals with incompletely defined biological activity necessitates such studies since the least adequately understood characteristics of many of these chemicals is their potential toxicity to man at his most vulnerable stage of development--conception to birth. While the more immediate transplacental effects such as abortion or teratogenic changes are relatively well studied, the often more insidious and potentially more harmful lifetime effects have received little attention. The great difficulty in correctly ascribing gestational origin to lesions in the human population which are subtle or do not become apparent until later in life makes laboratory and epidemiological investigations in this area essential.

Transplacental toxicity of selected environmental chemicals in mice. Chemicals investigated include Zearanol, Zearalenone, Mirex, Kepone, Hexabromobiphenyl (6-BB), Hexachlorobiphenyl (6-CB), Tetrachlorobiphenyl (4-CB), DMBA, 3-Methylcholanthrene, and Benzo(a)pyrene. The reproductive capacity and other postnatal toxicities following treatment with these chemicals were determined. In addition, the fetotoxic and/or teratogenic dose has been established for each of these compounds in the CD-1 mouse. For example, marked behavioral changes were observed in offspring from 4-CB treated (32 mg/kg/day) pregnant mice by two to three weeks after birth. These contract studies are done to support existing programs in the Laboratory of Environmental Toxicology. The purpose of the contract is two-fold; to identify environmental chemicals that have potential developmental toxicity emphasizing reproductive tract lesions; and to identify chemicals that are toxic and need to be studied from a mechanistic viewpoint by Intramural scientists.

Effects of DBCP on male reproductive function in mice. Dibromochloropropane (DBCP) has been shown to be mutagenic and carcinogenic in bacteria and experimental animals. DBCP has been extensively used in agriculture and linked to an increased incidence of male sterility among workers in processing plants. Sterility was associated with low sperm counts and a concomitant elevation of plasma gonadotrophins as well as incidence of testicular germinal aplasia from which recovery is uncertain. DNA repair in premeiotic spermatogenic cells of CD-1 mice was induced by a single i.p. administration of DBCP; in contrast no unscheduled DNA synthesis was induced in spermatozoa irrespective of dose. The exact mechanism of the action of DBCP with respect to germ cell damage is unknown. However, we speculate that dehydrobromination of DBCP might result in a reactive aliphatic epoxide which could then react with cellular macromolecules, especially germ cell DNA.

Effects of kepone on male reproductive function in mice. Kepone, a widely used pesticide, has a significant effect on testicular, epididymal and body weights of male rats. Sperm production was eliminated at the high dose (100 ppm). At 30 ppm, only body weight and cauda epididymal weights were significantly reduced. No recovery of any parameter was observed at the end of a three week post-treatment period. These data indicate an effect of Kepone on male reproductive organ weights and on sperm production, and suggest the need to examine in human males exposed to Kepone and to assess potential deficiencies in sperm fertilizing capacity using the heterologous *in vitro* test system.

B. Data extrapolation to man and risk estimation

Diethylstilbestrol-exposed mouse offspring as a model for similarly exposed humans. Many of the genital tract lesions observed in mice exposed prenatally to DES have been observed in comparably exposed humans. A good example of the utility of such studies is the report of retained testes in male mice derived from DES-treated mothers 2 years before a similar observation was reported in man.

Quantitation of chemical teratogenicity relative to maternal toxicity as a possible model for predicting relative human risk. When percent malformations are expressed as a function of percent of the LD-50 dose, a family of curves are produced which distinguishes quickly those chemicals which are teratogenic at doses well below maternal toxicity. Thus, a more reliable reference point than simply dose is established. The relationship between percent malformations and observable toxicity, such as CNS depression, is also being defined. Analyzing data concerning the teratogenicity of three anticonvulsant agents (trimethadione, diphenylhydantoin, and ethosuximide), it is readily apparent that the greatest clinical risk is associated with the use of trimethadione; the least with ethosuximide.

Testicular compartment model of pharmacokinetic and adaptive processes which aids interspecies comparisons. In the male gonads, factors which modify toxicity include the pharmacokinetic parameters governing the absorption, distribution, activation and detoxification of toxicants; covalent binding to macromolecules; and DNA damage as well as DNA repair of damaged germ cells. All of these factors are being studied in our laboratory at the present time. The male germ cells are protected by a biological barrier comparable to that which retards the penetration of chemicals to the brain; permeability constants for the two are nearly identical. Toxication and detoxication processes are present in both the seminiferous tubule and interstitial cellular compartments. The balance of toxication-detoxication processes apparently favors the germ cells; detoxification reactions are relatively more abundant in the seminiferous tubules. Unscheduled DNA repair has been demonstrated in spermatogonia and spermatocytes; spermatids and sperm lack DNA repair capability. The DNA repair capcity associated with spermiogenic cells appears to be dose-dependent and saturatable. Understanding the pharmacokinetic characteristics of the blood-testis barrier, toxification and detoxification mechanisms as well as DNA repair systems in male gonads will allow a better understanding of species comparison, of reproductive and genetic toxicity, and increase the reliability of extrapolating laboratory animal data to man and estimating human risk.

COLLABORATIVE RESEARCH

NIH CONTRACTS

Dr. McLachlan and Dr. Lucier of the Laboratory of Organ Function and Toxicology are contracting with the Wisconsin Alumni and Research Foundation to determine the transplacental toxicity of selected environmental chemicals.

COLLABORATIVE RESEARCH WITH ACADEMIC COMMUNITY AND GOVERNMENTAL AGENCIES

There are numerous examples of collaborative research projects at the local, national and international level. Laboratory scientists have acknowledged the resources and expertise concentrated in nearby universities. Moreover, we have not hestitated to establish the collaborative efforts necessary to develop quality intramural programs with scientists throughout the world.

Dr. Dixon has continued a productive collaboration with Dr. Sergio Fabro of the George Washington University. Dr. Fabro has been advisor to the Experimental Teratogenesis Workgroup which is concerned with mechanisms of teratogenesis.

Dr. Carter has collaborated with Dr. P. Goodfellow, Genetics Laboratory, Oxford University, England, regarding investigations of chromosomal proteins in fused cell mouse hybrids.

Dr. Hall initiated collaborative studies with the University of North Carolina to investigate effects of therapeutic agents (such as chemotherapy, radiation therapy, and treatment of infertility) on human male reproduction.

Dr. Korach is involved in several collaborative projects involving studies with DES. He is initiating a project with Dr. Ed Cook and Dr. Jack Keply of the Research Triangle Institute concerning the synthesis of ring-tritiated DES. A study of DES analogs and stimulation of DNA synthesis in the rat uterus is also being done in collaboration with Dr. Jack Gorski, University of Wisconsin. A collaborative project with Dr. Keith Baucom, PCR, Gainsville, Florida, concerning the synthesis of fluorinated DES is another of Dr. Korach's involvements.

Dr. Lee is collaborating with Dr. Harry Tyrer at Becton Dickinson Research Center on a project involving a fluorescence laser cell sorter system. The system was applied to study and quantitate 3-OH and 9-OH benzo(a)pyrene (BP) metabolites in testicular cells. Distribution patterns of BP metabolite in various spermatogenic cell types, and interstitial cells are also being studied.

Dr. Lee is studying differential DNA damage induced by chemical mutagens in cells growing in a modified Selye's granuloma pouch. This study is being done with Dr. Gerhard Zbinden of the Institute of Toxicology and University of Zurich in Switzerland.

Dr. McLachlan is involved in collaborative research with the Institute of Toxicology and Pharmacology, University of Wurzburg, Germany; Department of Obstetrics/Gynecology, Duke University Medical Center; Department of Anatomy, University of Colorado Medical School; and Department of Cooperative Medicine, Bowman-Gray School of Medicine. These collaborative projects involve a detailed exploration of transplacental toxicity of DES and other hormonally-active chemicals.

Dr. Sanyal is collaborating with Dr. R. E. Smith, Department of Microbiology and Immunology, Duke University, in the development of methods for continuous culture of rat embryos.

Drs. Dixon, Lee, and McLachlan are also involved in the US-USSR collaborative research programs in environmental health concerning transplacental toxicity and chronic toxicity.

NATIONAL AND INTERNATIONAL PROGRAMS: SYMPOSIA ORGANIZED/COMMITTEE APPOINTMENTS, ETC.

Another important indicator of peer approval and scientific relevance of current Laboratory programs is the frequency that LET scientists organize and participate in "state of the art" symposia and are asked to serve on various committees attempting to provide meaningful directions in environmental health research. Descriptions of representative examples for the Laboratory are given below:

Dr. Dixon has a large number of committee and program assignments which augment and are relevant to the NIEHS mission such as Chairman of the Committee on Environmental Pharmacology of ASPET, Committee on Women in the Workplace organized by NIOSH, Project Director of the teratogenic and reproductive effects areas of the NIEHS Energy-Related Research Program, US-USSR Problem II Coordinator of the Joint Committee for Health Cooperation-Environmental Health involving chronic organ toxicity, Council of the Pan-American Medical Association Section on Environmental Health Sciences, Council for Agricultural Science and Technology, and the Toxicology Review Panel of WHO's Expanded Programme of Research Development and Research Training in Human Reproduction. His recent appointment as Assistant to the Director for International Programs involves Dr. Dixon in the NIEHS' international activities including cooperative agreements with foreign countries, ad hoc international activities in response to particular scientific problems, temporary employment of foreign scientists, and informal exchanges of scientists (and technicians). This assignment encompasses current agreements between the US and the Soviet Union, Japan, Egypt, Italy, and the

World Health Organization (WHO) as well as future cooperative activities which will be developed. In addition, Dr. Dixon has been co-organizer of several Target Organ Toxicity Symposia in cooperation with the Society of Toxicology. Proceedings of this symposia are published in _Environmental Health Perspectives_ and include symposia on liver, kidney, lung, gonads, development, nervous system, and cardiovascular system.

Dr. McLachlan is a member of the DHEW Task Force on DES toxicity and has been advisor to NIOSH and the FDA on the toxicity of estrogens.

Dr. McLachlan has organized a symposium on "Estrogens in the Environment" sponsored by the NIEHS. Drs. Korach and Lamb served as members of the organizing committee for the symposium.

INFORMATION EXCHANGE

Communication of basic and applied information vital to environmental health problems is aided by establishing mechanisms for information exchange and by assuming editorial responsibilities. LET scientists are frequently asked to review manuscripts for journals oriented toward Biochemistry, Pharmacology, Toxicology, and Teratology. Dr. Dixon is on the Editorial Boards of _Environmental Health Perspectives_, _Toxicology and Applied Pharmacology_, _Journal of Pharmacology and Experimental Therapeutics_, _The Encyclopedia of Pharmacology and Therapeutics_, _Journal of Environmental Sciences and Health_, _Journal of Environmental Pathology and Toxicology_, and _Journal of Toxicology and Environmental Health_.

TRAINING PROGRAMS

Environmental health is a new and demanding research area that is undergoing rapid change and growth. Consequently, there is a growing need for training to ensure adequate numbers of qualified and dedicated researchers in environmental health research. The Laboratory of Environmental Toxicology recognizes this need and our scientists are encouraged to participate in a wide variety of training activities, including accepting adjunct appointments at nearby universities, supervising of graduate student research, developing graduate courses in environmental health, and participating in the Fogarty Visiting Program.

Dr. Hall is an Adjunct Assistant Professor at the University of North Carolina School of Medicine, Department of Obstetrics and Gynecology.

Dr. Harris is a member of the UNC Cancer Center.

Laboratory scientists have also been active in the training of graduate students. Graduate students are working in the laboratories of Drs. McLachlan, Dixon, and Korach.

Drs. Hall, McLachlan, and Korach have lectured at UNC and/or Duke in areas of their research expertise. Dr. McLachlan has also lectured at the Bowman-Gray School of Medicine. Dr. Korach has presented lectures at the Endocrine Research Center in Madison, Wisconsin, and the VA Medical Center in Minneapolis, Minnesota.

Dr. McLachlan was an invited speaker at the NIH Science Writers' Seminar in Bethesda, Maryland. At this training session for science writers, Dr. McLachlan presented a talk entitled "Toxification or Detoxification of Environmental Chemicals."

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70001-03 LET

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Teratogenicity of Antiepileptic Drugs and Chemically Related Compounds

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Kao	Visiting Fellow	LET	NIEHS
OTHER:	R. L. Dixon	Laboratory Chief	LET	NIEHS

COOPERATING UNITS (if any)

Columbia Hospital for Women, George Washington University

LAB/BRANCH
Laboratory of Environmental Toxicology

SECTION
Experimental Teratogenesis Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	2	2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Clinical observations have strongly implicated a number of antiseizure agents as human teratogens. The embryopathic potential of three classes of anticonvulsants: oxazolidinediones, hydantoins, and succinimides have been evaluated using the CD-1 mouse as the animal model. Estimated adult lethality curves were used as the basis for selecting doses for teratogenic investigations. Several dose levels with no observable maternal toxicity were evaluated. Following intraperitoneal administration, all the drugs were embryotoxic and produced not only intrauterine growth retardation, but also a range of malformations. Treatment on days 8-10 of pregnancy resulted in an increased incidence of congenital abnormalities in the skeletal and cardiovascular systems of the offspring while treatment on day 11-13 of pregnancy produced primarily cleft palate. In addition to their common teratogenic action, these compounds share a common chemical nucleus, namely a five membered imide ring. The finding that the three representative compounds are embryopathic strongly suggests a structure-teratogenic activity relationship. Upon comparing the relative teratogenicity of each anticonvulsant with respect to their relative risk to the dam, it was found that the teratogenicity of oxazolidinediones was significantly greater than that of the hydantoins and succinimides.

PROJECT DESCRIPTION

METHODS EMPLOYED: Lethalities were estimated in non-pregnant female CD-1 adult mice following three consecutive, daily i.p. administrations of the selected anticonvulsants. Lethality curves were obtained by analysis assuming an underlying probit model using the SAS probit analysis computer program. Dams were treated by single, i.p. injection of the test compounds on days 8, 9, and 10 or 11, 12, and 13 of pregnancy (plug date = day 1). The highest dose of the test chemicals used in embryotoxic and teratogenic evaluation was selected to be within the 95% confidence limits of the estimated adult LD_{01}. Methylcellulose solution (0.5% w/v) was the vehicle and appropriate controls were studied in parallel. On day 18 of pregnancy, the animals were sacrificed and laparotomy performed. Uteri were removed and the number of live, dead, and resorbing conceptuses recorded. Each live fetus was weighed and examined for external and internal abnormalities. Fetal heads were fixed in Bouin's solution and examined by Wilson's free hand slicing technique. Visceral examinations were performed by dissection with the aid of a stereomicroscope using the techniques outlined by Staples. Sketetal examinations were carried out after the decapitated and eviscerated fetuses were cleared and stained by the modified KOH-Alizarin Red S method.

MAJOR FINDINGS AND PROPOSED COURSE: The antiepileptic drugs tested are structurally related and are derivatives of cyclic imides. Thus, trimethadione, dimethadione, and paramethadione are derivatives of oxazolidinedione; diphenylhydantoin, ethotoin and mesantoin are hydantoins; and phensuximide, methsuximide and ethosuximide belong to the succinimide class of compounds. All the drugs and each of the basic chemical compounds produced embryotoxic and teratogenic effects. The malformation profile observed in the CD-1 mouse are similar to those described in humans following *in utero* exposure as in anticonvulsant therapy during pregnancy. Comparative computer analysis of the dose related increase in the incidence of malformations and adult lethality for each of the compounds indicated that the relative teratogenicity of the oxazolidinedione class of anticonvulsants was significantly greater than that of hydantoins and succinimides. These three classes of anticonvulsive agents share structural similarities and the finding that they are teratogenic suggests the possibility that the dysmorphogenic potential of these compounds might be a property of their common chemical nucleus.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In humans, anticonvulsant therapy during pregnancy has been associated with increased incidences of birth defects. However, such conclusions are largely based upon retrospective analysis of records, and the etiology of congenital abnormalities is often confounded by the influence of epilepsy *per se*. This study shows that selected anticonvulsant drugs are teratogenic in the CD-1 mouse, producing a spectrum of malformations which includes many of the abnormalities seen in humans. Thus, the CD-1 mouse appears to be a good animal model for use in studies of the relative teratogenicity of these agents. The comparison of relative teratogenic potential of these antiepileptic drugs may provide useful information for estimating the human risk-benefit relationship of anticonvulsant therapy during pregnancy. A common mechanism of action associated with the imide structure is suggested by the fact that each of these drugs share a common chemical moiety and all are embryotoxic and teratogenic.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70010-03 LET

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Study of Developmental Disorders During Organogenesis in a Model Embryo Culture System*

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. K. Sanyal	Reproductive Physiologist	LET	NIEHS
OTHER:	R. L. Dixon	Laboratory Chief	LET	NIEHS
	K. T. Kitchin	Staff Fellow	LOFT	NIEHS
	J. Allen	Staff Fellow	LEM	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Environmental Toxicology

SECTION
Experimental Teratogenesis Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To aid in the laboratory assessment of teratogens and the understanding of the molecular mechanisms underlying teratogenesis, an in vitro culture system for rat embryos has been developed. Rat conceptuses of pregnancy day 10 can be grown continuously for 96 hrs with extensive differentiation of major organs. Utilizing this embryo culture, nutritional and hormonal requirements for embryonic development are being studied, and the system's predictiveness for chemical teratogens is being tested. A microsomal enzyme activating system has been coupled to the embryo culture system which allows the detection of indirect acting teratogens.

(*Formerly, Study of Developmental Disorders in a Model of Embryo Culture During Organogenesis)

PROJECT DESCRIPTION

METHODS EMPLOYED: Rat embryos are cultured during organogenesis when they are most susceptible to teratogens. Conceptuses, dissected out of the mother on pregnancy days 10, 11 or 12, are cultured in heat inactivated homologous serum for 2-4 days at 38°C. Rotating bottles are used with a gas phase containing 10-40% O_2, 5% CO_2 and N_2. Growth promoting agents or teratogens are added to the culture medium to test their effect on embryonic differentiation. In vitro development of the embryo is evaluated using morphological (gross, histology and EM), biochemical (DNA, RNA, protein) and cytogenetic (karyotyping, sister chromatid exchange) parameters.

MAJOR FINDINGS AND PROPOSED COURSE: (1) Rat embryos of pregnancy day 10 can be grown for 96 hrs. in culture with 150-200 fold increase in DNA and protein content. The oxygen concentration in the gas phase must be gradually increased (10 to 40%) and medium changed. Extensive development of major organs occurs which is comparable to in vivo differentiation during the same period.

(2) Pregnancy day 10 rat conceptuses, cultured for 43-45 hrs., were highly sensitive to tetraethylene melamine (TEM) exposure. Embryonic development was abnormal at a dose level as low as 0.05 μg/ml medium. DNA and protein synthesis, differentiation of the neural tube, sensory organs, and somites were significantly affected by TEM.

(3) In vitro development of pregnancy day 11 rat conceptuses was unaffected by the addition of cyclophosphamide alone to the medium. However, addition of a combination of cyclophosphamide, NADPH and rat liver microsomes produced deleterious effects on embryonic development indicating metabolic conversion of cyclophosphamide into active components which presumably induce abnormal development.

The proposed course of work:

(1) Procedures for continuous in vitro growth and differentiation of embryos from preimplantation to organogenesis stages under more defined conditions will be developed. Nutritional and hormonal requirements for development and organogenesis of the embryo in vitro will be further elucidated.

(2) Effects of direct or indirect acting teratogens on the in vitro embryo development will be studied. Conditions for metabolic activation environmental agents by microsomal MFO enzymes as well as the maternal involvement in the production of actual teratogens will be explored in-depth.

(3) Studies relating to induction of embryonic chromosomal aberrations and sister chromatid exchange after BUdR labelling of chromatids will be expanded. Deleterious effect of BUdR on cellular differentiation and other cellular processes during organogenesis will be evaluated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Exposure to adverse environmental factors is presumably the major cause of developmental abnormalities in man. An *in vitro* system for culturing embryos during organogenesis, when the embryos are most susceptible to teratogens, provides an opportunity to study cellular and molecular processes associated with normal and abnormal development. This *in vitro* model will also be useful in predicting the teratogenic potential of environmental agents and in understanding the biological mechanisms by which teratogens induce birth defects.

PUBLICATIONS

Sanyal, M. K.: Secretion of progesterone during gestation in the rat. *J. Endocr.* 79: 179-190, 1978.

Sanyal, M. K.: Maturation and atresia of human oocytes *in vivo*. In Channing, C. P., Marsh, J. M. and Sadler, W. A. (Eds.): *Ovarian Follicular and Corpus Luteum Function*, Plenum Publishing Corp. 1979, pp. 321-323.

Sanyal, M. K. and Wiebke, E. A.: Oxygen requirement for *in vitro* growth and differentiation of the rat conceptus during organogenesis phase of embryo development. *Biol. Reprod.* 20: 639-647, 1979.

Sanyal, M. K.: *In vitro* development of rat conceptus during organogenesis in embryo: Relationship of embryo-placental growth and differentiation of changes in components of culture medium. *J. Exp. Emb. Morph.* (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70045-03 LET

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Molecular Mechanism of Androgen Mediated Gene Expression in Male Sex Accessory Glands

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. E. Harris	Senior Staff Fellow	LET	NIEHS
OTHER:	A. B. Silverberg	Staff Associate	LET	NIEHS
	D. B. Carter	Senior Staff Fellow	LET	NIEHS
	P. E. Mansson	Visiting Fellow	LET	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Toxicology

SECTION
Molecular Embryology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 4	PROFESSIONAL: 2	OTHER: 2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to study the mechanisms involved in androgen mediated gene expression in the rat prostate and the seminal vesicle. Protein synthesis patterns have been analyzed by 2D gel electrophoresis in castrate and testosterone stimulated prostate and seminal vesicle. A major group of secretory proteins in both organs is under androgen control. Both organs have a high concentration of poly(A$^+$)-mRNA which code in a wheat germ translation system for polypeptides which are similar to the in vivo secreted polypeptides. The two major poly(A$^+$)-RNA's from prostate (labeled β and γ) code for the subunits of the major secreted protein referred to as prostate binding protein. Likewise, rat seminal vesicle has two major poly(A$^+$)-mRNA's (labeled IV and V) which code for two major proteins. Double-stranded DNA copies (ds cDNA) of prostate β and γ poly(A$^+$)-mRNA and seminal vesicle poly(A$^+$)-mRNA for proteins IV and V were prepared using reverse transcriptase. With a variety of restriction enzymes, maps were prepared for the various ds cDNA's. Subsequently, the cDNA's for β and γ from prostate were cloned in the plasmid pBR322 utilizing the "tailing method."
(*Formerly, Teratocarcinoma System as a Model for Gene Expression in Normal and Abnormal Development)

PROJECT DESCRIPTION

METHODS EMPLOYED: Seminal vesicles and prostates were removed from mature CD rats. Seminal vesicle secretions were collected for preparation of purified proteins. Seminal vesicle proteins IV and V were purified by a combination of ion exchange chromatography and molecular sieve chromatography. Poly(A$^+$)-mRNA was prepared by affinity chromatography using oligo-dT cellulose and fractionated on 5-20% sucrose gradients in either 1% SDS or 70% formamide. The wheat germ *in vitro* translation system was used to characterize various poly(A$^+$)-mRNA fractions. ^{3}H-complementary DNA was prepared using reverse transcriptase. RNA-DNA hybridizations were carried out under standard conditions. Double stranded complementary DNA was again synthesized using reverse transcriptase. A variety of restriction endonucleases were employed to cleave the ds cDNA's prepared from both prostate and seminal vesicle. The fragments generated with the restriction enzymes were analyzed by neutral agarose gel electrophoresis. For the prostate, the ds cDNA's of β and γ poly(A$^+$)-mRNA were treated with S1 nuclease under conditions to cleave the single-stranded "hairpin" loop at the 5' end. The ds cDNA β and γ was then "tailed" with 50-200 dT residues using terminal deoxynucleotide transferase and annealed to plasmid pBR322 (previously tailed with dA at the single Pst I site). This chimeric plasmid was mixed with treated *E. coli* 1776. Tetracycline resistant colonies were selected (~370) and then tested on plates containing ampicillin (amp). One-hundred fifty amp sensitive colonies were then grown on millipore filters and tested by colony filter hybridization with ^{32}P-cDNA probes enriched for the individual prostate mRNA's (β and γ). Colonies giving strong positive hybridization signals on the filters are presently under investigation.

Laemmli SDS polyacrylamide slab gel electrophoresis has been utilized to analyze *in vivo* proteins and *in vitro* translation products. Two-dimensional electrophoresis has been performed. Agarose gel electrophoresis with methyl mercury hydroxide was used to characterize various RNA preparations.

MAJOR FINDINGS AND PROPOSED COURSE: When total poly(A$^+$)-mRNA from rat ventral prostate is analyzed by sucrose gradient centrifugation, a major peak of RNA (40% of the total) is observed at 10S (ribosomal RNA's were used as standards). This RNA, having a 10S sedimentation rate can be fractionated into a light and heavy side. The light side is enriched in poly(A)-mRNA referred to as γ mRNA, while the main heavy side is enriched in poly(A$^+$)-mRNA referred to as β mRNA. By denaturing agarose gel electrophoresis, the size of prostate β mRNA and γ mRNA are determined to be 520 nucleotides (NT) and 450 NT, respectively. γ mRNA codes in a wheat germ translation system for a polypeptide of 14,000 daltons, similar to one of the subunits of prostate binding protein (PBP). β mRNA codes, on the other hand, for two major polypeptides which are related to the major protein subunit of PBP.

Double-stranded complementary DNA (ds cDNA β and γ) to the prostate β and γ poly(A^+)-RNA was prepared using reverse transcriptase (synthetic genes). The ds cDNA β was separated from the ds cDNA γ by preparative agarose gel electrophoresis. The relative pure ds cDNA's to γ and β were then restricted with a variety of restriction enzymes and preliminary maps generated for the two prostate synthetic genes. A large batch of ds cDNA β and γ was synthesized (~2 μg), treated with S1 nuclease, tailed with terminal deoxynucleotide transferase, and cloned in the plasmid pBR322 (see methods). Four clones, were identified which had a high probability of containing either β or γ ds cDNA inserts by colony filter hybridization (see methods). One hundred ml cultures of these 4 clones were grown and the plasmids isolated from those clones are presently under investigation using a variety of restriction enzymes.

A similar approach is being applied to the major abundant poly(A^+)-mRNA from rat seminal vesicle. A major 11S poly(A^+)-mRNA has been identified which comprises 40% of the total poly(A^+)-mRNA in the seminal vesicle. This 11S poly(A^+)-mRNA appears as two major bands in agarose gel electrophoresis under denaturing conditions. The size of the two poly(A^+)-mRNA bands are 650 NT (referred to as $mRNA_{sv1}$) and 580 NT (referred to as $mRNA_{sv2}$). Poly(A^+)-mRNAs enriched for $mRNA_{sv1}$ code (wheat germ) for a polypeptide of 18,000 daltons, similar in molecular weight but slightly larger than the seminal vesicle secreted protein IV. Likewise, the $mRNA_{sv2}$ appears to code for a polypeptide of 14,000 daltons, again similar and slightly larger than the secreted protein V.

Double-stranded cDNA (ds $cDNA_{sv1}$ and $_{sv2}$) to poly(A^+)-$mRNA_{sv1}$ and $_{sv2}$ was prepared as described above. The two seminal vesicle synthetic genes were separated and preparatively isolated by gel electrophoresis using low melting agarose. The bands were removed, the agarose melted, and the DNA isolated by hydroxyapatide chromatography. Restriction maps were then generated for the synthetic genes SV^1 and SV^2. Very interestingly, it appears as if the structural parts of these two seminal vesicle genes may have considerable sequence homology.

In the future, the prostate β and γ clones will be further characterized. With these cloned DNA fragments, probes for specific regions of these genes will be developed. Similarly, the seminal vesicle ds cDNA to $mRNA_{sv1}$ and $mRNA_{sv2}$ will be cloned and probes developed for these genes. With characterized probes for these 4 rat androgen regulatable genes, the study of RNA precursors to these specific genes becomes possible. Also, detailed analysis of the structure and function of these genes in the chromosomal DNA becomes feasible with the availability these androgen specific gene probes.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A study of a wide variety of hormonally regulatable genes, both from a structural as well as functional point of view, should result in a more complete understanding of the detailed molecular aspects of gene action. With this information, we can perhaps better predict the effect of various environmental chemicals on the variety of steps involved in gene function.

PUBLICATIONS

Harris, S. E.: Basic mechanisms of ovarian function germ cells. Env. Health Persp. 24: 11-16, 1978.

Carter, D. B. and Harris, S. E.: Chromosomal proteins from the embryoid body/teratocarcinoma system. Dev. Biol. 69: 1-14, 1979.

Mansson, P. E. and Harris, S. E.: Complexity of poly(A)-containing heterogeneous nuclear ribonucleic acid from mouse embryoid bodies (OTT 6050). Biochemistry 18: 2073-2078, 1979.

Mansson, P. E., Silverberg, A. B., Tully, D. B. and Harris, S. E.: Purification of the major abundance class poly(A)-RNA from rat seminal vesicles. Nucleic Acid Research. (In press).

PROJECT DESCRIPTION

METHODS EMPLOYED: 8-12 Müllerian or Wolffian ducts from fetal mice (beginning at day 14 post-conception) are incubated in ^{35}S-methionine supplemented DMEM for two hours on agar blocks. After incubation, tissue extracts are prepared by 1% SDS-8 M urea-1% DTT and 2% NP-40 and 160,000 xg centrifugation in a Beckman airfuge. Final analysis is done by isoelectric focusing and SDS-Laemmli gel electrophoresis and fluorography.

MAJOR FINDINGS AND PROPOSED COURSE: The course of differentiation of the embryonic mouse genital tract was followed by high resolution two dimensional (2D) protein mapping of Müllerian and Wolffian duct proteins. Of the 400 proteins detectable in a single pattern, between 5 and 30 proteins are either developmental stage specific or sex specific. The sex differences tend to increase with fetal age whereas the stage specific differences show no developmental trend. The effects of diethylstilbestrol on the protein synthetic patterns will be assessed by the same technique.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The developing mouse genital tract has been proposed as a model system for the study of the effects of DES on differentiation and subsequent pathology of the mammalian genital tract. The technique is capable of utilizing the small amounts of tissue available to yield physical information on up to 500 different proteins containing methionine. The assessment of the effects of DES on normal mouse genital tract differentiation as expressed by changes in subpopulations of detectable proteins in the 2D maps may provide a sensitive assay for the carcinogenic potential of other environmental agents.

PUBLICATIONS

Carter, D. B. and Harris, S. E.: Chromosomal proteins from the embryoid body/teratocarcinoma system. Dev. Biol. 69: 1-14, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70060-06 LET

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effect of Prenatal Exposure to Foreign Chemicals on Genital Tract Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. A. McLachlan	Head, Transplacental Toxicology Group	LET	NIEHS
OTHER:	K. S. Korach	Senior Staff Fellow	LET	NIEHS
	Y. Suzuki	Visiting Fellow	LET	NIEHS
	D. B. Carter	Senior Staff Fellow	LET	NIEHS
	L. Levy	Research Chemist	EBCB	NIEHS
	M. Hogan	Mathematical Statistician	BB	NIEHS
	M. Luster	Immunologist	EBCB	NIEHS
	J. C. Lamb, IV	Developmental Pathologist	LET	NIEHS

COOPERATING UNITS (if any)

Bowman-Gray School of Medicine
Environmental Biology and Chemistry Branch, NIEHS
Environmental Biometry Branch, NIEHS
Univ. Colorado Medical Center
Cancer Research Center, UNC
University of Wurzburg, Wurzburg Germany

LAB/BRANCH
Laboratory of Environmental Toxicology

SECTION
Transplacental Toxicology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.6	2.8	1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range goals of this project are: (1) to evaluate the effects of prenatal exposure to environmental chemicals on the subsequent reproductive capacity of the offspring; (2) to investigate the mechanisms involved in the production of subfertility in mammals as a result of their in utero exposure to foreign chemicals; (3) to assess the transplacental carcinogenic potential of these compounds; (4) to study the physiologic disposition and metabolism of these compounds in the pregnant animal and fetus; (5) to study chemico-biological interactions of transplacental toxicants, with special emphasis on structure-activity relationships; (6) to determine if prenatal exposure to environmental agents can alter the cytoplasmic receptors for steroid hormones in reproductive tract tissues; (7) to develop and utilize organ culture systems to study the effects of environmental chemicals on the fetal ovary and reproductive tract in vitro; and (8) to evaluate the above animal models as predictors of human response. Special attention is given to diethylstilbestrol (DES).

PROJECT DESCRIPTION

METHODS EMPLOYED: The most sensitive measurement of female gonadal function is her total reproductive capacity as determined by repetitive forced breeding techniques. In order to assess the function status of the female gonad, these techniques were coupled with the determination of ovarian periodicity, quantity, and morphology of ova obtained from the female reproductive determinations of the various follicular classes. Pharmacological, biochemical, physiologic, and morphologic procedures were used which included polyacrylamide gel electrophoresis, radioimmunoassay, histochemistry, autoradiography, inverse isotope dilution analysis and chromatography, microdissection, and scanning electron microscopy. Organ culture techniques were developed to maintain explants of fetal ovaries and reproductive tracts.

MAJOR FINDINGS AND PROPOSED COURSE: The synthetic estrogen, diethylstilbestrol (DES), is a common environmental chemical currently used as a livestock growth promotor and gynecologic medication. Experiments in our laboratory have demonstrated that prenatal exposure to DES adversely affects the reproductive capacity of the male and female offspring. Continuation of these studies has shown that such prenatal exposure results in a low incidence of female genital tract neoplasms including vaginal and uterine adenocarcinoma, squamous cell tumors of the vagina and ovarian tumors. A common non-neoplastic lesion of the DES-treated mouse was squamous metaplasia of the uterus. This abnormality was determined to require a secondary stimulus by estrogen at puberty for its manifestation. Stromal hyperplasia of the vagina, cervix and uterus has raised the question of the role of this tissue component in the observed lesions; the demonstration of uterine and cervical stromal sarcomas in prenatally DES-treated mice further emphasizes the importance of studies on stromal-epithelial interactions during abnormal development of the genital tract. Similar lesions could not be produced following prenatal exposure to the steroidal estrogen, 17β-estradiol. Differential fetal protein binding of DES and estradiol may help explain these results. Bioavailability at many levels may be a determining factor in the transplacental toxicity of hormonally-active xenobiotics.

Scanning electron microscopy (SEM) of the genital tract of these females has revealed surface ultrastructural abnormalities which may precede neoplastic changes; SEM may provide a tool for early detection of neoplastic change in the affected tissues. This technique was also used to demonstrate hormone-related changes in genital tract tissue during the estrous cycle and with the onset of puberty. The latter study coupled with determinations of estrogen receptor levels and enzyme activities suggests that hormone responsiveness is manifested earlier in the vagina than in the uterus. Alterations in the location of squamocolumnar junction as well as vaginal polyps have been determined by this approach. Both male and female offspring of DES-treated mice had abnormal immune function.

In continuation of studies with male offspring from DES treated mice, the fate of Müllerian duct remnants in DES treated males was determined and the role of these tissues in prostatic lesions evaluated. These remnants were shown to respond to estrogen with typical Müllerian features. Müllerian remnants were observed in treated male offspring at any stage of development. Alterations in these tissues were, in part, related to changes in estradiol and Müllerian inhibiting Factor (MIF). Additionally, SEM studies of the male genital tract have been undertaken to establish sensitive morphological parameters of cellular abnormalities. Most abnormalities observed in the genital tracts of exposed men and women have been duplicated in our mouse model. Additional alterations seen in the mouse may be predictive for the human. In both male and female offspring, attempts will be made to establish biochemical/morphological markers for genital tract lesions. For example, the SDS protein profile of the secretions of the prostate and seminal vesicles of DES mice were shown to be altered. Similar altered patterns in the uterine luminal fluid were consistent with altered morphology.

The distribution, metabolism and structure-activity relationships of DES in perinatal systems have continued. Oxidative metabolites of DES (e.g. β-dienestrol and ω-hydroxy DES) were identified in the mouse fetus and neonate exposed to ^{14}C-DES. Moreover, studies on the bioactivation of DES have shown the non-extractable binding of radioactivity to DNA and protein after incubation of ^{14}C-DES with several activating systems including one derived from target tissue, the mouse uterus. This same activating system utilizing peroxidase was determined in the hamster kidney, a non-genital target tissue for DES. The estrogenic activities of a series of DES metabolites and analogs were determined. Results suggest that DES metabolism follows alternative pathways resulting in metabolites which retain estrogenicity or those in which such activity is absent. These studies have been expanded with special emphasis on the biological significance of potentially activated metabolites; such data should aid in generalizations to other classes of estrogenic environmental chemicals. In addition, studies of DES metabolism in target/non-target tissues and in cell culture are being continued. For example, it was shown that peroxidase, an enzyme inducible in estrogen target tissue, is able to metabolize DES to its major metabolite, β-dienestrol. Fluorinated derivatives of DES have been made to help assess the role of metabolism in toxicity.

Studies in organ culture have shown that DES can alter normal differentiation of the genital tract _in vitro_. These studies will be continued to evaluate the role of organ/organ and cell/cell interactions in genital tract development. Thus, DES has been shown to alter the action of MIF on the _in vitro_ differentiation of the Müllerian ducts. Two-dimensional gel electrophoretic maps of the protein changes during organogenesis of the female genital tract have been developed to aid in an understanding of the molecular events which determine normal or abnormal differentiation of this system. Also, experiments utilizing the separation and recombination of stroma and epithelium of DES treated fetal reproductive tracts have been undertaken to determine the role of such tissue interactions in DES induced genital abnormalities.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Although many compounds are continuously introduced into our environment, few of them have been examined for their potentially toxic effect on reproduction and development. Virtually nothing is known about the effect of prenatal exposure to common drugs and chemicals on the postnatal development of the offspring. The fact that no division of oocytes occurs postnatally in man or laboratory rodents makes the process of oogenesis especially susceptible to chemical intervention during the prenatal period. However, the effects of such *in utero* drug exposure may not become evident until much later in the animal's life when sexual maturity is reached. Given the possibility of long-term genetic damage to the developing oocyte or transplacental carcinogenetic changes in the reproductive tract, reduced fertility in the offspring may be only the most obvious consequence of prenatal exposure to environmental chemicals.

PUBLICATIONS

Metzler, M. and McLachlan, J. A.: Oxidative metabolites of diethylstilbestrol in the fetal, neonatal, and adult mouse. Biochem. Pharmacol. 27: 1087-1094, 1978.

Metzler, M. and McLachlan, J. A.: Is diethylstilbestrol bioactivated by peroxidase mediated oxidation? J. Env. Path. Toxicol. 1: 531-533, 1978.

Luster, M. I., Faith, R. E. and McLachlan, J. A.: Modulation of the antibody response following *in utero* exposure to diethylstilbestrol. Bull. Environ. Contam. Toxicol. 20: 433-437, 1978.

Lamb, J. C., IV, Stumpf, W. E., Newbold, R. R., and McLachlan, J. A.: Transitional changes in the surface epithelium of the cycling mouse vagina, cervix, and uterus: Scanning electron microscopic studies. Biol. Reprod. 19: 701-711, 1978.

Metzler, M. and McLachlan, J. A.: Peroxidase-mediated oxidation, a possible pathway for metabolic activation of diethylstilbestrol. Biochem. Biophys. Res. Comm. 85: 874-884, 1978.

McLachlan, J. A., Metzler, M., and Lamb, J. C., IV: Possible role of peroxidase in the diethylstilbestrol-induced lesions of the syrian hamster kidney. Life Sciences 23: 2521-2524, 1978.

Metzler, M. and McLachlan, J. A.: Proposed nomenclature for diethylstilbestrol metabolites. J. Environ. Pathol. Toxicol. 2: 579-582, 1978.

Metzler, M. and McLachlan, J. A.: Oxidative metabolism of diethylstilbestrol and steroidal estrogens in relation to their fetotoxicity. In, Role of Pharmacokinetics in Prenatal and Perinatal Toxicology, ed. D. Neubert, H.-J. Merker, H. Nau, and J. Langman, pp. 157-164, Georg Thieme Publishers, Stuttgart, 1978.

McLachlan, J. A., Korach, K. S., and Metzler, M.: Bioavailability as a Determinant in the Transplacental Toxicity of Diethylstilbestrol. In, Neubert, D., Merker, H.-J., Nau, H., Langman, J. (Eds.): Role of Pharmacokinetics in Prenatal and Perinatal Toxicology. Stuttgart, Georg Thieme Publishers, 1978, pp. 147-155.

McLachlan, J. A.: Transplacental effects of diethylstilbestrol in mice. Proceedings of the Conference on Perinatal Carcinogenesis, NCI Monograph Series. (In press).

Lucier, G. W., Davis, G. J., and McLachlan, J. A.: Transplacental toxicology of the polychlorinated and polybrominated biphenyls. Proceedings of the 17th Hanford Biology Symposium. Developmental Toxicology of Energy-Related Pollutants. Ed. D. D. Mahlum, M. R. Sikov, P. L. Hackett, and F. D. Andrew, pp. 188-204, Department of Energy Symposium Series 47, 1978.

Luster, M. I., Faith, R. E., McLachlan, J. A., and Clark, G. C.: Immunological effects following in utero exposure to diethylstilbestrol in mice. Annals of Internal Med. (In press).

Luster, M. I., Faith, R. E. and McLachlan, J. A.: Effect of in utero exposure to diethylstilbestrol on the immune response in mice. Tox. Appl. Pharm. (In press).

Metzler, M. and McLachlan, J. A.: Diethylstilbestrol metabolic transformation in relation to organ specific tumor manifestation. Archives Toxicol. (In press).

Tilson, H. A., Davis, G. J., McLachlan, J. A., and Lucier, G. W.: The effects of polychlorinated biphenyls given prenatally on the neurobehavioral development of mice. Environ. Res. (In press).

Lamb, J. C., IV, Newbold, R. R. and McLachlan, J. A.: Evaluation of the transplacental toxicity of diethylstilbestrol with the scanning electron microscope. J. Toxicol. Environ. Health, 5: 599-603, 1979.

McLachlan, J. A., Baucom, K., Korach, K. S., Levy, L. and Metzler, M.: A novel fluorinated derivative of diethylstilbestrol. Steroids 33: 543-547, 1979.

Korach, K. S., Metzler, M. and McLachlan, J. A.: Diethylstilbestrol metabolites and analogs: new probes for the study of hormone action. J. Biol. Chem. (In press).

Suzuki, Y., Lamb, J. C., IV, and McLachlan, J. A.: Age-dependent morphological changes in the seminal colliculus in male mice: Light and scanning electron microscopic study. Anatomy and Embryology. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70065-03 LET

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Role of Chemical-Receptor Interactions in Reproduction and Transplacental Toxicity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. S. Korach	Senior Staff Fellow	LET	NIEHS
OTHER:	J. A. McLachlan	Head, Transplacental Toxicology Group	LET	NIEHS
	J. K. Haseman	Statistician	BB	NIEHS
	L. Levy	Chemist	EBCB	NIEHS

COOPERATING UNITS (if any)

University of Wurzburg
Biometry Branch, NIEHS
Environmental Biology and Chemistry Branch, NIEHS

LAB/BRANCH

Laboratory of Environmental Toxicology

SECTION

Transplacental Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MANYEARS: 2.7	PROFESSIONAL: 1.3	OTHER: 1.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The main objectives of this project are to determine whether DES is metabolized to biologically and hormonally active metabolites; to test the hypothesis that certain chemicals are "transplacental toxicants" due to their relative binding to plasma/receptor proteins particularly alpha-fetoprotein; to investigate some of the biochemical mechanisms which contribute to effects of prenatal exposure of mice to hormonally active environmental chemicals to determine the molecular locus of transplacental toxicity using structure-function relationships of different environmental chemicals; and to determine biochemical markers for transplacental toxicity. These objectives are approached using refined biochemical techniques of hormone receptors and hormone action. The basic physiological effects on hormone synthesis and hormone levels will be studied using chemical extraction techniques and radioimmunoassays. The carcinogenic nature of hormonally active environmental chemicals will be studied in vivo.

PROJECT DESCRIPTION

METHODS EMPLOYED: Hormonal effects were studied with receptor binding techniques including saturation binding, competition studies, Scatchard plot analysis, sucrose gradient centrifugation, glucose oxidation/utilization, RNA polymerase enzyme activities, extraction of nuclear DNA polymerase enzymes and DNA synthesis quantitation, polyacrylamide gel electrophoresis, dual isotope labeling and protamine sulfate precipitation. Biochemical studies employed spectrophotometric enzyme assays and routine chemical isolation and extraction techniques.

MAJOR FINDINGS AND PROPOSED COURSE: Prenatal administration of DES results in female offspring of two distinct groups: those with hormonally nonresponsive uteri and those that are hyperstimulated. To understand why some uteri were not hormonally responsive, the concentration of estrogen receptors in these animals were examined. Characterization of uterine nuclear estrogen receptors in the mouse has indicated two forms of the receptor (analogous to Type I, II reported in rat). Presently, the best experimental conditions for studying these receptor forms, are being determined. Results have shown that those animals in the non-responsive group have significantly lower levels of estrogen receptor. In order to distinquish the age at which this difference in receptor level occurs between DES treated and control animals, receptor levels in 5 through 40 day old animals have been measured. These studies suggest that at 5 days of age this pattern of receptor differences is already apparent. Cytosol receptor concentrations in vaginal tissue were not significantly different from controls. Further experiments with the uterus and vagina from DES exposed mice will determine which step in the mechanism of hormone action is altered. The difference in cytosol receptor levels could not be explained by differential accumulation of receptors in the nucleus since assays of nuclear receptor in these same tissues showed no differences.

Receptor differences in control and DES treated offspring were also found in studies demonstrating the responsiveness of the receptors to estrogen administration. The mouse uterus possesses a second translocation of hormone receptor complex to the nucleus after exposure to hormone. Compounds with poor estrogenic potency lack the ability to elicit this second nuclear peak. The role of this event in estrogen action in the mouse reproductive tract, with particular regard to the actions of hormonally active environmental chemicals, is being studied since DES treated animals have an altered pattern of receptor depletion/replenishment. Receptor resynthesis, RNA polymerase activities, DNA polymerase activities and glucose oxidation/utilization is also being investigated.

In order to determine whether the metabolism of DES resulted in biologically active or inactive metabolites, certain DES metabolites and analogs were tested for estrogenic activity using both an in vivo bioassay and an in vitro receptor binding assay. Results of these studies showed good correlation between the biochemical and bioassay data. Compounds such as DES-epoxide or catechol-DES were associated with reasonable receptor binding and biological activity; while certain metabolites, e.g. β-dienestrol or ω-hydroxy dienestrol, showed weak receptor interactions and poor estrogenicity. This indicates that the metabolism of DES does not result in complete inactivation. The exception to these results was indanyl-DES, a DES analog and possible metabolite, which shows receptor binding comparable to DES, but was 20-25 times less biological active. These studies have suggested a mechanism of altered/differential clearance of these analog receptor complexes from the target cell nucleus. Studies of receptor and plasma binding activities, particularly to alphafetoprotein, of various DES analogs and metabolites will be continued to determine the structural site of chemicals exhibiting hormonal and/or carcinogenic actions. A unique DES derivative containing fluorine atoms has been synthesized and its potential carcinogenic and hormonal activity is being tested.

Binding studies are being expanded to allow the potential hormonal activity of selected environmental chemicals to be determined in this model. Complete hormone action involves the ability of the hormone to influence resynthesis of its receptor; only some of these DES compounds showed this hormonal property which was related to their biological efficacy. Additional studies will be undertaken to determine if these compounds can also stimulate progesterone receptor synthesis.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The association of *in utero* DES exposure and reproductive tract cancer in human subjects is well documented. Recent development of a mouse model to study these effects will allow this problem to be more fully investigated. The objectives of this project are to define the roles of receptor protein-chemical interactions and the biochemical mechanisms associated with the toxicologic responses observed in the reproductive tract following *in utero* exposure to hormonally active environmental chemicals.

Since knowledge of *in utero* environmental chemical effects on the reproductive system of the offspring is so limited, these studies will help recognize other clinical and biomedical problems which may arise from exposure to environmental compounds. Determining the mechanism by which these chemicals act will help in the development of reasonable safeguards.

PUBLICATIONS

Korach, K. S. and Ford, E. B.: Estrogen action in the mouse uterus: An additional nuclear event. *Biochem. Biophys. Res. Comm.* 83: 827-833, 1978.

Korach, K. S.: Estrogen action in the mouse uterus: Characterization of the cytosol and nuclear receptor systems. *Endocrinology* (In press).

Korach, K. S., Metzler, M. and McLachlan, J. A.: Diethylstilbestrol metabolites and analogs: New probes for the study of hormone action. J. Biol. Chem. (In press).

McLachlan, J. A., Baucom, K., Korach, K. S., Levy, L. and Metzler, M.: A novel fluorinated derivative of diethylstilbestrol. Steroids 33: 543-547, 1979.

McLachlan, J. A., Korach, K. S. and Metzler, M.: Bioavailability as a Determinant in the Transplacental Toxicity of Diethylstilbestrol. In Neubert, D., Merker, H.-J., Nau, H., Langman, J. (Eds.): Role of Pharmacokinetics in Prenatal and Perinatal Toxicology. Stuttgart, Georg Thieme Publishers, 1978, pp. 147-155.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70068-02 LET

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Correlative Morphological Studies of Abnormal Cell Differentiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. C. Lamb, IV	Developmental Pathologist	LET	NIEHS
OTHER:	J. A. McLachlan	Head, Transplacental Toxicology Group	LET	NIEHS
	Y. Suzuki	Visiting Fellow	LET	NIEHS
	K. S. Korach	Senior Staff Fellow	LET	NIEHS

COOPERATING UNITS (if any)

Bowman Gray School of Medicine
U.N.C. Cancer Research Center

LAB/BRANCH
Laboratory of Environmental Toxicology

SECTION
Transplacental Toxicology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies are designed to: (1) evaluate the influence of in utero exposure to environmental toxicants (for example, diethylstilbestrol) on fine structure of the male and female reproductive tracts; (2) study the influence of environmental chemicals on cell differentiation and cell surface ultrastructural features; (3) develop and utilize models which will determine the cell surface features associated with preneoplastic and neoplastic transformation by scanning and transmission electron microscopy and correlative histology, (4) employ histochemistry to identify and localize morphological markers to assess the cellular or subcellular responsiveness to foreign agents, and (5) determine with autoradiography, the cellular and subcellular localization of steroid hormones in normal or abnormal reproductive tract tissues.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The scanning electron microscope has proven to be a very sensitive tool for the evaluation of cell surface changes associated with normal and abnormal cell differentiation. The pathological evaluation of lesions found with the scanning electron microscope in reproductive tract tissue is made by subsequent processing for light microscopy so that classic morphological criteria may be used to classify genital tract abnormalities. When in vitro systems such as tissue culture specimens are evaluated, the samples are first studied by phase contrast microscopy, interference contrast microscopy or immunofluorescence microscopy, and cell surface changes can be correlated to histological changes on a cell-to-cell basis. Correlative or concurrent transmission electron microscopy or histochemistry have also been employed. Histochemical methods have also been developed as highly sensitive assays to be used in conjunction with biochemical or enzymological assays. Autoradiography of steroids can indicate whether hormones are processed similarly in normal or transformed tissues.

MAJOR FINDINGS AND PROPOSED COURSE: Previous studies in our laboratory have demonstrated that in utero exposure of the CD-1 mouse to diethylstilbestrol (DES) may serve as a model for prenatal toxicity. Prenatal exposure DES is associated with male and female genital tract abnormalities in mice.

Studies in our laboratory have demonstrated that scanning electron microscopy (SEM) provides a tool for the detection of early neoplastic changes. The surface ultrastructural features of the lumen of the female mouse genital tract was evaluated during the estrous cycle and during development in normal CD-1 mice. The hormone-dependence of fine structural features of the cell surface was demonstrated in ovariectomized, hormone-treated females where various characteristics of intact animals were experimentally induced. Subsequent studies on DES-treated mice indicate that cell surface features are directly related to abnormal cell differentiation. Changes in the cell surface are correlated with alterations in the histological features of DES-exposed offspring. Histochemical and high resolution electron microscopic studies also play an integral role in the evaluation of the mechanism of DES-toxicity. Enzyme histochemical studies are ongoing to determine the precise location of the metabolic enzymes, peroxidase, which may activate DES to a toxic intermediate.

Autoradiographic studies are being initiated to determine the ability of transformed cells to process steroid hormones in a normal manner. The evaluation of steroid hormone localization at critical time points can demonstrate an association between cell transformation and a disruption of normal hormone-receptor interactions.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Cell surface changes are thought to be important in neoplastic transformation. However, the influence of in utero exposure to environmental toxicants on the subsequent development of cell surface ultrastructure has not been evaluated. The transplacental toxicity of DES in the CD-1 mouse is being used as a model for morphological changes associated with exposure to toxic chemicals early in development. Neoplasia may be one endpoint of abnormal differentiation or altered development in these animals. To properly evaluate pathological lesions, however, comprehensive investigations into morphological changes in normal development and aging are critical. Correlative studies of steroid hormone action and multilevel morphologic changes in their target tissues will help understand the normal and abnormal biology of this important organ system.

PUBLICATIONS

Lamb, J. C. IV, Newbold, R. R., Stumpf, W. E. and McLachlan, J. A.: Transitional changes in the surface epithelium of the cycling mouse vagina, cervix, and uterus: Scanning electron microscopic studies. Biol. Rep. 19: 701-711, 1978.

Lamb, J. C., IV, Newbold, R. R. and McLachlan, J. A.: Evaluation of the transplacental toxicity of diethylstilbestrol with the scanning electron microscope. J. Toxic. Envr. Hlth. 5: 599-603, 1979.

McLachlan, J. A., Metzler, M. and Lamb, J. C., IV: Possible role of peroxidase in the diethylstilbestrol-induced lesions of the Syrian hamster kidney. Life Sciences 23: 2521-2524, 1978.

Lamb, J. C., IV and Ingram, P. I.: Drying of biological specimens for scanning electron microscopy directly from ethanol. SEM/1979/I. (In press).

Suzuki, Y., Lamb, J. C., IV, and McLachlan, J. A.: Age-dependent morphological changes in the seminal colliculus in male mice: Light and scanning electron microscopic study. Anatomy and Embryology. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70070-03 LET

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Transplacental Pathology of Environmental Chemicals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. L. Dixon	Laboratory Chief	LET	NIEHS
OTHER:	O. A. Meyer	Visiting Scientist	LET	NIEHS
	J. Kao	Visiting Fellow	LET	NIEHS
	E. E. McConnell	Pathologist	EBCB	NIEHS

COOPERATING UNITS (if any)
Environmental Biology and Chemistry Branch
Division of Laboratory of Animal Resources, Duke

LAB/BRANCH
Laboratory of Environmental Toxicology

SECTION
Developmental Pathology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 3	PROFESSIONAL: 1	OTHER: 2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is designed to evaluate the effect of environmental chemicals on offspring of female rats subjected to totigestational exposure. Selected biochemical, histological and behavioral parameters are monitored for transplacental pathology. Three chemicals, methyl-n-butyl ketone, enflurane (ethrane), and ethchlorvynol (Placidyl), were studied initially. Postnatal effects observed were generally associated with changes in blood chemistry parameters and behavioral tests. However, these effects, although statistically significant, often lacked a dose or temporal relationship which complicated the assessment of their biological importance. There was no significant histopathology or carcinogenicity associated with any of these treatments. A more recently initiated experiment which sought to determine the possible postnatal effects associated with prenatal benzene exposure was terminated due to a mycoplasma infection outbreak in the animal colony.

PROJECT DESCRIPTION

METHODS EMPLOYED: Mated female Fischer rats are exposed to selected chemicals throughout gestation by appropriate routes beginning on day 0 of gestation and continuing through day 20. A dose associated with no natural toxicity and causing minimal overt effects at birth is used. Selected physiologic, biochemical, histological and behavioral observations are made at each of five stages of development: newborn, weanling, puberty, five months and 18 months of age. The organ systems monitored include: liver; kidney; hematopoietic; sensory; central and peripheral nervous system; reproductive; skeletal muscle; immunological; and cardiovascular. Standard techniques and assay procedures are employed.

MAJOR FINDINGS AND PROPOSED COURSE: To date totigestational studies on three chemicals have been initiated.

(A.) Methyl-n-butyl ketone (MBK). When administered by inhalation at 500 ppm, the only effect of MBK noted was a 12 to 24 hour delay in parturition. At 1000 ppm the exposed dams were smaller than corresponding controls which correlated with a decrease in litter size. While birth weights of treated and control animals were comparable, the growth rate of offspring from treated females was decreased at all stages of development to adulthood. Female offspring of treated mothers showed retarded sexual maturation as determined by time of vaginal opening. Shock avoidance acquisition and retention were deficient in female offspring but not in males. Female offspring of treated animals were significantly more active when tested on an activity wheel. No other lesions have been detected physiologically, histologically, or biochemically.

(B.) Enflurane (Ethrane). Enflurane was administered by inhalation at 0.15% for six hours per day for 21 days starting at day 0 gestation. Serum fluoride was monitored for three consecutive days, day 0, 1 and 2. Extrapolation from these results suggest that a plateau level of approximately 1.7×10^{-5}M would be reached by day 5 or 6. Enflurane treated females delivered a larger percentage of small litters (less than seven pups) than did controls. Birth weight and subsequent growth rate of offspring of treated females were comparable to controls. Clinical chemistry tests in weanling pups of treated females revealed an increased creatinine phosphokinase activity and elevated triglyceride level. Behavioral tests showed no significant differences between offspring from treated and control females. The differences in clinical chemistry observed in weanling pups were not evident at pubertal or adult stages. All other parameters were comparable to controls.

(C.) Ethchlorvynol (Placidyl). Ethchlorvynol was administered daily by oral intubation throughout gestation at 6 doses ranging from 0 (olive oil) to 80 mg/kg. No alteration in maternal weight or fetal development was apparent.

Clinical chemistry determinations in weanling animals revealed an elevated blood glucose and a decrease in phosphorus, creatine phosphokinase, SGOT, SGPT, and in [Na-(Cl + CO_2)] balance. No differences in behavior have been observed. At puberty female offsprings from treated animals showed a significant increase in phenobarbital sleeping time, but only at the low treatment dose; male offsprings showed little or no effects. Clinical chemistry determination in treated animals at puberty revealed an elevated blood glucose and a decreased BUN at the 20 mg/kg dose. Triglycerides were elevated at the 80 mg/kg dose. All other parameters were comparable to control.

(D.) Benzene. Benzene was administered via inhalation at 100 ppm for six hours each day for 21 consecutive days following positive evidence of mating. The experiment was terminated after 60 days because of an outbreak of mycoplasma at the institute. No significant results were noted.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The human population now has a lifetime exposure to an increasingly large number of chemicals with incompletely defined biological activity. Among the least adequately understood characteristics of many of these chemicals are their potential toxicity to man at his most vulnerable stage of development--conception to birth. While the more immediate transplacental effects such as abortion or teratogenic changes are relatively well studied among these agents, the often more insidious and potentially more harmful lifetime effects have received little attention. The great difficulty in correctly ascribing gestational origin to lesions in the human population which are subtle or do not become apparent until later in life makes laboratory and epidemiological investigations in this area essential. Hopefully laboratory studies will yield data which can be used to identify chemicals in the environment which produce adverse transplacental changes at levels to which the human population is exposed.

Postnatal toxicity is also of special interest from a more basic standpoint. Toxic effects of gestational chemical exposure can often be related to a specific developmental stage of the embryo. Thus, a gestational "window", limited to three or four weeks for most laboratory animals, is present during which a biologically unique organism is present each day. Special susceptibilities to chemical toxins likely accompany this developmental process. Thus, gestation presents a molecular kalidoscope with ever-changing targets for chemical toxins. Such chemical-biological interactions might be reliable predictors of birth defects as well as more subtle postnatal effects.

PUBLICATIONS

Peters, M. A., Hudson, P. M., and Julgelske, W., Jr.: The acute toxicity of methylglyoxal in rats: The influence of age, sex, and pregnancy. Ecotoxicol. Environ. Saf. 2: 369-374 (1978).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70080-06 LET

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Study of Toxic Effects of Environmental Chemicals on Spermatogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	I. P. Lee	Pharmacologist	LET	NIEHS
OTHER:	R. L. Dixon	Laboratory Chief	LET	NIEHS
	R. Shoji	Visiting Associate	LET	NIEHS
	J. Nagayama	Visiting Fellow	LET	NIEHS
	J. Bend	Visiting Scientist	LP	NIEHS

COOPERATING UNITS (if any)

Laboratory of Pharmacology

LAB/BRANCH
Laboratory of Environmental Toxicology

SECTION
Germ Cell Toxicity Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.6	3.5	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies seek to better assess the effects of environmental agents on spermatogenesis and male reproductive capacity. Mechanisms of toxicity are studied and new approaches to toxicity testing are proposed and validated. In addition, selected environmental agents are tested for reproductive toxicity. The following studies are ongoing: (1) defining the role of germ cell DNA repair as an indicator and modifier of toxicity; (2) modeling the various factors which affect testicular toxicity; and (3) investigating the toxication-detoxication of polycyclic hydrocarbons in gonadal tissues using in vitro and perfused testis preparations. To date, selected mutagens, trace metals, TCDD, nitrous oxide, Freon 22, and dibromochloropropane (DBCP) have been studied. Alkaline elution analysis of germ cell DNA appears to be a reliable indicator of damage and repair as well as a predictor of toxicity. The inter-relationship of the blood-testis barrier, toxication-detoxication reactions, and DNA repair have been described. These various studies contribute to an increased understanding of mechanisms of toxicity and better laboratory tests as well as more reliable extrapolation of laboratory data to man and improved estimates of human risk.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: (A) DNA Repair. Effects of 1,2-dibromo-3-chloropropane (DBCP) on spermatogenic cell DNA was investigated by labeling spermatogonial DNA of 15 to 30 day old mice with repeated i.p. administrations of tritiated thymidine. Following DBCP administration, the spermatogenic cells were isolated from minced tubules by 0.1% collagenase digestion. DNA molecular size was estimated by alkaline elution analysis elution profiles. *In vitro* and *in vivo* DNA repair was also studied by pretreating rodents with DBCP, followed by tritiated thymidine and hydroxyurea. Spermatogenic cell DNA radioactivity was subsequently determined.

(B) *In vivo* assessment of fertility. Fertility was assessed using the serial mating technique. Fertility profiles were drawn relating infertility to the type of spermatogenic cell affected.

(C) Studies of polycyclic hydrocarbon activating and deactivating enzyme systems. Benzo(a)-pyrene hydroxylase (AHH), epoxide hydrase (EH), glutathione S-transferase (GSH-ST), and cytochrome P-450 were measured in both testicular and prostatic tissues. The various factors affecting the specific activities of these enzymes in testes and prostate glands studied included hypophysectomy, pretreatment with tetrachlorodibenzo-p-dioxin (TCDD) and the role of gonadotropins (LH and FSH).

(D) *In vitro* perfusion of rat testis. Rat testis was quickly excised and the testicular artery cannulated with a 80-100 micron glass capillary tube attached to polyethylene tubing. Oxygenated Kreb-ringer bicarbonate solution containing 3% albumin (BSA fraction IV) was used to perfuse testis at 20 ml g^{-1} . hr^{-1}. Benzopyrene-7,10-^{14}C (3.6 x 10^{-6} M) was perfused, and BP metabolites and glutathione diol conjugates were determined by a combination of differential solvent extraction, thin layer chromatography, high pressure liquid chromatography (HPLC), and liquid scintillation spectrometry. Viability of the perfused testis was assessed by glucose consumption, and the concentration of glycolytic intermediates in the perfusate and effluent.

(E) *In vitro* fetal organ cell aggregation patterns. Pregnant Sprague-Dawley rats on day 14 of gestation were exposed to a single X-irradiation of 25, 50, 100, and 200 R. Immediately after treatment, fetal brains were excised and monodispersed cells obtained by collagenase treatment. Five million cells in minimum essential medium with Eagle's base (pH 7.3-7.5) were cultured in a gyratory shaker at 37°C up to 48 hrs. The fetal brain cell aggregation patterns and cell aggregate volumes were measured after 24 and 48 hrs of incubation. Cell surface changes as well as the site of cell interaction were observed with both transmission and scanning electron microscopy.

MAJOR FINDINGS AND PROPOSED COURSE: (A) DBCP has been shown to be mutagenic and carcinogenic in bacteria and experimental animals. DBCP has been extensively used in agriculture and linked to an increased incidence of male sterility among workers in processing plants. Sterility was associated with low sperm counts and a concomitant elevation of plasma gonadotrophins as well as testicular germinal aplasia from which recovery is uncertain at the present time. DNA repair in spermatogenic cells of CD-1 mice was induced by a single i.p. administration of DBCP. Unscheduled DNA synthesis was assayed in isolated premeiotic germ cells and sperm from adult male mice at various times after DBCP doses of 80, 100, 120, and 130 mg/kg. DNA repair synthesis in isolated premeiotic cells after 100 and 120 mg/kg of DBCP was significant and increased linearly during the first 8 hrs following treatment. In the highest dose treatment group (130 mg/kg), unscheduled DNA synthesis increased linearly for the first 4 hrs and then remained constant. In contrast to premeiotic spermatogenic cells, no unscheduled DNA synthesis was induced in spermatozoa irrespective of dose. The exact mechanism of the action of DBCP with respect to germ cell damage is unknown. However, we speculate that dehydrobromination of DBCP might result in a reactive aliphatic epoxide which could then react with cellular macromolecules, especially with the premeiotic germ cell DNA.

(B) There was no statistically significant dominant lethal effects associated with the mature spermatozoa, spermatids, spermatocytes following a MTD dose of DBCP (100 mg/kg). However, there was a decreased number of live implants associated with exposure of spermatogonia.

(C) Induction of AHH and EH specific activities in rat testes and prostate glands. A single oral dose of TCDD (10 μg/kg) induced AHH and cytochrome P-448 levels in liver (L), testis (T), and prostate gland (P) as a function of time in CD rats. The maximum induction of AHH activity and cytochrome P-448 in L, T, and P were 4, 1.5, and 150 and 1.4, 1.3, and 166 times control levels, respectively. After TCDD treatment, the optimum time for maximal induction of AHH activity in L, T, and P was 48, 16, and 16 hrs, respectively; the optimum time for induction of cytochrome P-448 in L, T, and P, was 48 hrs for each tissue. The induction of AHH and cytochrome P-448 in each of these tissues can be completely inhibited by pretreatment with either actinomycin-D or cycloheximide suggesting induction of de novo synthesis of specific proteins. The huge increase in AHH activity in the prostate gland following TCDD was particularly noteworthy. The induced AHH and cytochrome P-448 levels in P were about 0.5 and 0.1 of that observed in normal hepatic microsomes. The induction of AHH activity and cytochrome P-448 may be involved in the etiology of prostatic cancer.

Effects of hormones on the regulation of AHH, EH, and glutathione-S-transferase (GSH-ST) activities and P-448 levels in testis of hypophysectomized rats were studied. This was done to determine whether testicular AHH, EH, GSH-ST activities and P-450 levels are affected by FSH, LH, and testosterone (T). Sham-operated and hypophysectomized (HYPOX) male rats were studied. Forty days after HYPOX, rats received 3 daily injections of LH, FSH, T or LH plus FSH (100 μg each) or saline. Rats were killed 5, 10, and 15 days after the last treatment. In HYPOX rats, testicular AHH, EH activities, and P-450 content were significantly reduced (20-70% of sham-operated control values) at all time points while GSH-ST activity was unchanged. In contrast, testicular AHH, EH, and P-450 were significantly increased in all LH treatment groups, but not after FSH or T treatment. The greatest LH effect (5-14 fold) was found 15 days after treatment. The results suggest that LH-stimulated induction of testicular AHH, EH, and P-450 contents is associated with the interstitial cell compartment.

(D) Since toxic effects of arene oxides on male germ cells are affected by various pharmacokinetic factors not represented in cell free systems, the isolated perfused testis (IPT) was utilized to better study testicular metabolism of chemicals. The testis was perfused with BP-4,5-oxide-^{3}H via the testicular artery. BP-4,5-diol and glutathione conjugates were formed at the rate of 2.3, 7.7, and 14.9 pmol min^{-1} g testis^{-1} and 26.8, 75.3, and 103.4 pmol min^{-1} g testis^{-1}, when the substrate concentration was 1.3, 3.6, and 50 X 10^{-6} M. The metabolism of benzo(a)pyrene (BP) was studied in control and tetrachlorodibenzo-p-dioxin (TCDD) treated rats. The testicular artery was perfused with 7,10-^{14}C-BP. The BP concentration was 3.5 x 10^{-6}M and the rate of perfusion was 20 mls hr^{-1} . gm testis^{-1}. BP metabolites were extracted from both the perfusate and the testis and subjected to HPLC analysis. Analysis of the perfusates indicated that 4-11% of the BP was metabolized by the testis in 60 minutes; a metabolic rate equivalent to 3-7 nmoles . g testis^{-1}. A variety of metabolites including phenols, quinones, and dihydrodiols were identified. Metabolites in the perfusate were 9,10-diol (1.4%), quinones (1.1%), 4,5-diol (0.44%), 3-OH (0.46%), 9-OH (0.22%), 7,8-diol (0.18%); metabolites in the testis were 9,10-diol (1.4%), 7,8-diol (0.5%), 3-OH (0.46%), 9-OH (0.25%), quinone (0.24%) and 4,5-diol (0.1%). The amount of 7,8-diols and 3-OH in the testis was 1.5 to 3 fold greater and the amount of 4,5-diol and quinones were 4 to 5 fold less than measured in the perfusate. Pretreatment of rats with TCDD (10 μg/kg) significantly increased the production of quinones, 3-OH, 9-OH, and 7,8-diols in both the perfusate and testis. The relatively higher 9,10-diol in the testicular tissue suggests that testicular EH may have lower Km for 9,10-epoxide. The significance of the high levels of 9,10-diol in the testicular tissue and the perfusate needs further study.

(E) In search of a simplified *in vitro* test system to predict both physical and chemical teratogens, the effects of total body maternal X-irradiation on cell aggregation patterns of enzymatically isolated fetal brain cells were studied. Maternal whole body X-irradiation on day 14 of gestation results in multiple birth defects including microcephaly and/or hydrocephalus. Thus, pregnant Sprague-Dawley rats were exposed on day 14 of gestation to a single dose of 25, 50, 100 or 200 R. During the first 4 hrs, neither size nor shape of the brain cell clusters from control and experimental groups differed. However, at 24 and 48 hrs, the size of control cell aggregates were significantly larger than those of X-irradiated animals after doses of 100 and 200 R. In contrast, no differences in the cell aggregation pattern were observed in animals in groups receiving lower doses of X-irradiation. Preliminary results of scanning electron microscopic studies revealed that cell aggregation patterns after prenatal exposure to X-irradiation were significantly different from controls with respect to both size and shape of aggregates.

In vitro brain cell aggregation in control groups was associated with development of membrane filopodia and a subsequent complex interaction of membrane filopodia between the cells and cell aggregates. In contrast, X-irradiation (>50 R) prevented the development of membrane filopodia with coincident inhibition of interactions between aggregates. Transmission electron microscopic studies revealed that X-irradiated groups exhibited a large sinusoidal space within the cell aggregates with large numbers of degenerative cells along the inner surface of the sinusoidal space. Furthermore, at higher magnification, cell surface microvilli, rough endoplasmic reticulum, and tight cell-cell junctions observed in controls were lacking in the X-irradiated groups. These results suggest that perturbation of morphogenetic fetal brain cell interactions during differentiation are associated with malformations. Preliminary results suggest that membrane factors which influence cell aggregation might be a sensitive early target for teratogens. Ongoing studies will determine if changes in cell aggregation can be induced by chemical teratogens.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Toxicological studies of a target organ, such as the testis, seek to elucidate qualitatively and quantitatively the toxic effects of a chemical on that organ. The ultimate objective is to assess the toxic effects of a chemical in laboratory animals and extrapolate the pertinent experimental data to man. To accomplish these objectives, one must consider the main factors which may influence and modulate the toxic effects of chemicals in the organ. In the male gonads, such modifying factors are the pharmacokinetic parameters governing the absorption, distribution, activation and detoxification of toxicants; covalent bindings to macromolecules; and DNA damage as well as DNA repair of damaged germ cells. All of these factors are being studied in our laboratory at the present time. Short term tests of reproductive and developmental toxicity are also sought.

DNA repair is a sensitive indicator of chemically damaged spermatogenic cells. In addition, the repair capability of male germ cells affords the organism some degree of protection against toxic agents. Chemicals such as benzo(a)-pyrene are both activated and detoxified by enzymes in the testis; enzyme activities differ between the interstitial cellular and seminiferous tubular compartments. The study of the role of biotransformation in the testis involves in vitro subcellular fractions, perfused organ, and whole animal approaches. Fetal organ aggregation patterns appear to be an indicator of developmental abnormalities. The behavior of monodispersed mouse brain cells following maternal whole body X-irradiation and selected chemicals is being studied.

Improved short term tests are essential to modern toxicology. Prediction of reproductive toxins and teratogens is especially difficult. Understanding the pharmacokinetic characteristics of the BTB, toxification and detoxification mechanisms as well as DNA repair systems in male gonads will allow a better understanding of species comparison, of reproductive and genetic toxicity, and increase the reliability of extrapolating laboratory animal data to man and estimating human risk.

PUBLICATIONS

Lee, I. P. and Dixon, R. L.: Mutagenicity, carcinogenicity and teratogenicity of procarbazine. Mut. Res. 55: 1-14, 1978.

Lee, I. P. and Dixon, R. L.: Factors influencing reproduction and genetic toxic effects on male gonads. Envir. Health Pers. 24: 117-127, 1978.

Lee, I. P., Sherins, R. J., and Dixon, R. L.: Evidence for induction of germinal aplasia in male rats by environmental exposure to boron. Toxicol. Appl. Pharmacol. 45: 577-590, 1978.

Schmid, B., Lee, I. P. and Zbinden, G.: DNA-repair processes in germ-cells demonstrated in ejaculated sperms of rabbits treated with methylmethane sulfonate. Arch. Toxicol. 40: 37-43, 1978.

Mukhtar, H., Lee, I. P., Fourman, G., and Bend, J. R.: Epoxide metabolizing enzyme-activities in rat testes. Postnatal development and relative activity in interstitial and spermatogenic cell compartments. Chem-Biol. Inter. 22: 153-165, 1978.

Mukhtar, H., Lee, I. P. and Bend, J. R.: Glutathione S-transferase activities in rat and mouse sperm and human semen. Biochem. Biophys. Res. Comm. 83: 1093-1098, 1978.

Mukhtar, H., Philpot, R. M., Lee, I. P., and Bend, J. R.: Developmental aspects of epoxide metabolizing activities in adrenals, ovaries, and testes of rat. Department of Energy Symposium Series, Oak Ridge, Tenn. pp. 89-104, 1978.

Lee, I. P., and Zbinden, G.: Differential DNA damage induced by chemical mutagens in cells growing in modified Selye's granuloma pouch. Exp. Cell Biol. 47: 92-106, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70085-02 LET

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Development of In Vitro Models for Assessing Reproductive Toxicity*

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Hall	Research Physiologist	LET	NIEHS
OTHER:	R. L. Dixon	Laboratory Chief	LET	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Toxicology

SECTION
Germ Cell Toxicity Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Heterologous (human sperm/hamster egg) in vitro fertilization was used to assess fertilizing capacity of human males. These studies identified certain individuals as subfertile who have routinely determined normal sperm number, motility and morphology. This approach demonstrated that the usual parameters for semen analyses are unsure predictors of male subfertility; only the most extreme semen abnormalities reliably indicate subfertility. Thus, the in vitro penetration of laboratory animal ova by human sperm appears to be an improved technique to assess human fertilization potential. The epidemiologic application of this noninvasive, fast and relatively inexpensive technique is being explored.

(*Formerly, Development of In Vitro Models for Toxicological Testing in Reproduction)

PROJECT DESCRIPTION

METHODS EMPLOYED: In addition to the need for greater emphasis on animal research regarding reproductive effects of environmental hazards, usable animal models are urgently needed to identify the causative factors for the decline in human male fertility. These studies were designed to test the utility of the interspecies fertilization system to assess human infertility, and to identify subtle changes in sperm fertilizing capacity possibly related to environmental agents. Methods used to test human fertility and determine reproductive effects of a potent environmental pollutant are described.

A. The heterologous in vitro fertilizing capacity of sperm from fertile men was compared to the fertilizing capacity of sperm from the male partner of 43 couples referred to an infertility clinic. Each semen sample was evaluated for sperm number, motility and morphology one hour after collection. Sperm were prepared so that each fertilizing dish contained approximately equivalent numbers of motile sperm. Ova for fertilization were collected from Golden hamster oviducts by superovulation; they were denuded and incubated in vitro with sperm suspensions from either fertile or suspected infertile men. After three hours, ova were examined by phase-contrast microscopy for morphological evidence of fertilization. Ova observed to have at least one dispersed sperm head or pronucleus with adjacent sperm tail in the cytoplasm were considered fertilized. Electron microscopy accompanied the light microscopic evaluation.

B. Utilizing CD-Fisher rats, the effect of continuous dosing of Kepone on several reproductive parameters was studied. Seven week old rats were fed Kepone (0, 10, 30, 100 ppm) in their diet for 50 days. Testicular and cauda epididymal weights, body weights, epididymal sperm number, sperm motility and percent abnormal sperm morphology were determined at the end of the treatment period. Recovery from Kepone treatments was evaluated 21 days after the end of the 50 day treatment.

MAJOR FINDINGS AND PROPOSED COURSE: The in vitro fertilization system using hamster ova with human sperm can discriminate between highly fertile and poorly fertile men with similar sperm motility and morphologic characteristics. The mean fertilization rate was 60% for the proven fertile donors and 31% for men suspected of having fertility problems. All donors fertilized more than 20% of the ova while sperm from 44% of the possible infertile subjects fertilized fewer than 20%. Since no donor sperm fertilized fewer than 20% of the ova in any test, men whose sperm samples fertilized fewer than 20% were arbitrarily defined as subfertile. There appears to be a poor correlation between the routinely determined parameters of sperm number, motility or morphology and fertilizing capacity of human sperm. Of the parameters evaluated, percent motility corresponded more closely with in vitro fertilization results, although nearly a third of the unproven subjects within normal range for this parameter were judged subfertile. The correlation between in vitro fertilization and semen parameters improved as the number of semen parameters increased. The degree of subfertility decreases with increasing number of normal parameters. However, even when all five parameters are within normal limits, about a third of these patients are apparently subfertile. These experiments have led to the conclusions, important to both human and animal reproductive research, that (1) the heterologous (interspecies) in vitro fertilization test system using hamster ova is a more accurate predictor of human sperm fertilization potential than standard semen analyses, and (2) there exist defects or deficiencies in human sperm, possibly of environmental etiology, which profoundly inhibit or diminish their capacity to fertilize. This system can be made available for fertility screening following accidental exposure of men to toxic agents. The relationships between occupational chemical exposure and in vitro fertilization results of male partners of infertile couples needs to be intensively investigated.

Evaluation of test data indicate a significant effect of high (100 ppm) Kepone exposure on testis, epididymal and body weights of male rats. Sperm production was eliminated at the high dose. At 30 ppm, only body weight and cauda epididymal weights were significantly reduced. No sperm abnormalities were seen in any dose group. Three weeks after the end of treatment, the four parameters examined were still significantly different from controls at the high dose; no sperm was formed and transported into the cauda epididymis during the three week period. These data indicate an effect of Kepone on male reproductive organ weights and on sperm production, and suggest the need to examine human males exposed to Kepone and to assess potential deficiencies in sperm fertilizing capacity using the heterologous in vitro test system.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The homologous and heterologous fertilization models provide the means to establish the relationships of test chemicals in animals to occupational agents in humans regarding loss of reproductive capacity. Further, an approach to study specific mechanisms by which various toxic chemicals exert their effect on sperm function now exists.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70120-08 LET

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Hepatotoxicity of Hormonally-Active Chemicals*

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. W. Lucier	Acting Lab Chief	LET/LOFT	NIEHS
OTHER:	W. Powell-Jones	Visiting Fellow	LET/LOFT	NIEHS
	E. Lui	Visiting Fellow	LET/LOFT	NIEHS
	C. A. Lamartiniere	Senior Staff Fellow	LET/LOFT	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Toxicology/Laboratory of Organ Function and Toxicology

SECTION
Hepatic and Endocrine

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.4	3.0	2.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long-range plan of this project to study and understand changes in hepatic function following exposure to environmental agents emphasizing effects of hormonally-active chemicals. These studies are defining the liver as a target organ for estrogens by characterizing cytosolic and nuclear estrogen-binding proteins and correlating the presence of receptors with estrogen mediated induction or repression of protein synthesis. The functional biochemical components of estrogen action in adult liver appear to be imprinted during a critical neonatal period by endogenous hormones. The imprinting of sex-dependent hepatic protein synthesis is also evaluated in these studies using a variety of enzyme markers. The pituitary-hypothalamic-hepatic axis appears to regulate the ontogeny of hepatic metabolic competence and the mechanisms involved are investigated in whole animal and culture systems. Alterations in the sex differentiation of hepatic enzymes (specific activities and kinetic constants) are studied in relation to susceptibility to hepatotoxic agents.

*Metabolism and Binding of Steroids and Xenobiotics in Developing Systems

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Steroid receptors in rat liver cytosol were measured by a variety of assay procedures utilizing dextran-coated charcoal, ammonium sulfate and sucrose gradients. Total and available binding sites were measured. Binding constants and amounts of receptors were determined by Scatchard Plots. Nuclear receptors and translocation processes were studied using liver and uterine slices and cell free systems. Substrate specificities were evaluated by competitive binding techniques. Individual estrogen binding proteins were examined by placing estrogen-receptor complexes on Sephadex G-75 columns. The importance of the pituitary and gonads in regulating synthesis of liver estrogen receptors was studied using intact, hypophysectomized and castrated rats. Rats were gonadectomized as adults or castrated neonatally. Neonatal castrates in some cases, were given replacement therapy with testosterone propionate (TP) or diethylstilbestrol (DES). These studies also examined the effects of hormonally-active environmental agents on microsomal enzyme activity. Specific chemicals included in the studies are DES, selected PCB isomers, methadone and certain steroid hormones. Chemicals were administered prenatally (p.o. administration to pregnant rats on selected days of gestation) or postnatally (s.c. administration to newborn rats during the first week of life). Some animals were subjected to various surgical procedures (i.e. hypophysectomy and/or castration) prior to being treated with testosterone propionate, DES or dihydrotestosterone (days 56-63, s.c.). Following treatment, enzyme activities were studied in the immature (20 days old) or mature (63 days old) rat, to determine the effect of the various treatments on enzyme development.

In some experiments, adult rats which had been treated neonatally with hormonally-active chemicals, were administered cadmium chloride and effects on hepatic enzymes were determined.

MAJOR FINDINGS AND PROPOSED COURSE: Estrogen Action in Liver. Adverse side effects of estrogenic compounds on the liver are receiving increasing attention. Certain of these estrogen-induced changes could reflect a direct liver-hormone interaction dependent upon the presence of estrogen receptors in the liver cells. Our studies demonstrate that liver cytosol fractions from both male and female rats contain estrogen-binding components possessing criteria assigned to receptor proteins. These criteria include a finite binding capacity together with a high affinity and binding specificity for estrogens. Interestingly, male liver contains an additional estrogen-binding protein(s) distinct from the receptor. The physiological significance of this protein(s) is under investigation. These binding proteins appear to be under pituitary-hypothalamic control. Both male and female estrogen-receptor complexes undergo translocation into liver nuclei. In contrast to uterus, two conformational forms of estrogen-receptor complexes are evident in both male and female liver nuclei. The presence of receptors indicates that the liver is a target organ for estrogens. The study of hepatic estrogen-receptor interactions and the consequences of this estrogen action is clearly of importance in determining the impact of estrogenically-active chemicals on liver function. To further investigate the sex differentiation of hepatic estrogen binding protein(s). $(^3H)E_2$ labeled cytosol from male or female rats was applied to a Sephadex G-75 column and each fraction was assessed for bound radioactivity. The elution profile of labeled proteins shows a species (peak I, MW > 75, K) common to both sexes, which binds $(^3H)E_2$ at comparable levels to that of the specific estrogen receptor. In addition, 3 other species (peaks II, IV and V) are present in both sexes, with male levels being quantitatively greater than female. Also present is a species (peak III) found only in male cytosol. Competition studies demonstrate differences in steroid specificity for individual proteins. The elution profile of labeled cytosol from immature male liver resembled that of the adult female. Gonadectomy of adult male and female rats has no effect on the protein profiles. However, male rats which were castrated one day after birth and sacrificed as adults exhibit a disappearance in peak III and a marked reduction in the level of peak V. Hypophysectomy of adult male rats also results in the disappearance (feminization) of peak III and reductions approaching female levels in peaks II, IV and V. Hypophysectomy of female rats results in partial masculinization of the protein profile; appearance of peak III and increases in peaks II, IV and V. These studies show that qualitative and quantitative differences in estrogen binding proteins contribute to the observed sex differences in levels of 4S binding. The maintenance of this sex difference is pituitary dependent and may be programmed at birth through hypothalamic-pituitary axis by neonatal androgen exposure. Further studies will examine the biological/biochemical response of the liver following administration of endogenous estrogens or estrogenically-active environmental agents. These studies will utilize in vivo systems as well as primary culture of isolated hepatocytes. Indicators studied will include the production of renin substrate, triglycerides, VLDL (very low density lipoproteins), glucose 6-phosphate dehydrogenase, and RNA polymerase. The quality and quantity of hepatic estrogen binding proteins will be manipulated by surgical procedures such as hypophysectomy and castration and by altering the normal neonatal imprinting of specific binding proteins. The age-dependent response of the liver will also be investigated. The long-term goal is to correlate receptor level and type with toxic responses of the liver to estrogens.

Hormonal Regulation of Hepatic Monoxygenase. Sex-related differences in the hepatic microsomal ethylmorphine N-demethylation activities were observed only in control mature rats; specific activities in males were 4-fold higher than in females. Administration of TP or DES to neonates resulted in the "feminization" (depression) of hepatic ethylmorphine N-demethylation activities of adult male rats without significantly altering the enzyme activities in either the mature female rats or immature rats of either sex. Furthermore, kinetic studies revealed neonatal "feminization" of Km values for the N-demethylation in adult male rats. Reduction in serum androgen levels of adult male rats was associated with the treatment of neonates with DES but not with TP. Reduction of hepatic ethylmorphine N-demethylation activity and cytochrome P-450 contents following cadmium treatment (2.0 mg/kg, ip.) was also age- and sex-dependent in the rat; marked reduction in enzyme activity was observed only in adult male rats. Adult male rats which had received TP or DES treatments during the neonatal period exhibited decreased sensitivity towards the hepatotoxic effects of cadmium; responses in the treated groups were similar to those of females. The sex-dependent response to cadmium is under pituitary-hypothalamic control and is associated with the amount of cadmium sensitive forms of cytochrome P-450 as demonstrated by synthesis/degradation studies on hepatic hemoproteins. The levels of cadmium sensitive forms appear to be imprinted during a critical period of neonatal development by androgens.

Administration of methadone to male neonates resulted in female type Km values for the hepatic monooxygenase system in the subsequent adult animal whereas V_{max} values were unchanged. This feminization of kinetic values appears to be related to a methadone-mediated depression of testosterone levels in neonates suggesting that methadone prevents the imprinting of the monooxygenase system. This idea was reinforced by the finding that simultaneous administration of testosterone and methadone to neonates resulted in normal sex differentiation of enzyme activity.

Recent evidence suggest that receptors in hepatic cytosol for polycyclic hydrocarbons are required for the induction of cytochrome P-448-catalyzed enzyme activities. It has been postulated that cytoplasmic receptor-ligand complex translocates to the nucleus in a manner analogous to that described for the steroid hormone receptor. To examine the mode of induction of hepatic enzyme activities by non-steroidal inducing agents, we have investigated the effect of hypophysectomy on the induction of hepatic monooxygenase activities by 3,4,3',4'-tetrachlorobiphenyl (4-CB) (3-MC type inducer) and 2,4,5,2',4',5'-hexachlorobiphenyl (6-CB) (phenobarbital-type inducer). Treatment of sham-hypophysectomized (Hx) rats with 4-CB (initiated at 12 days following surgery) resulted in a 30- and 3-fold increase in 7-ethoxyresorufin o-deethylation (ERD) activity and chtochrome P-448 contents, respectively. This inductive effect of 4-CB was reduced significantly in the absence of pituitary glands. Exposure of sham-Hx rats to 6-CB resulted in significant increases in hepatic ED activity and cytochrome P-450 levels; however, the magnitude of induction by 6-CB was not altered by prior-hypophysectomy (12 days). These data suggest that pituitary glands may play a role in the induction of cytochrome P-448-catalyzed reactions but not cytochrome P-450-dependent reactions. However, when the inductive effect of 4-CB was examined at 21 or 28 days following Hx, the extents of the induction of ERD activity and cytochrome P-448 contents were of the same order of magnitude as those detected in sham-Hx rats. The time-dependent restoration of normal hepatic responsiveness to 4-CB in Hx rats suggest development of compensatory or alternative mechanisms for cytochrome P-448 induction.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: During recent years it is becoming increasingly evident that the liver may be an toxicologically important target organ for estrogens. For example there are many adverse side effects of oral contraceptives that could be related to estrogen action in the liver. Some of these side effects are; increased incidence of 1) thrombosis, 2) heart attacks, 3) jaundice, 4) gall stones, 5) hypertension, and 6) benign hepatomas. The occurrence of thrombosis may be related to increased synthesis of plasma clotting factors; cardiovascular disease could be enhanced by increased hepatic synthesis of plasma triglycerides and certain lipoproteins; estrogen alters the hepatic transport of bile acids and bilirubin and therefore might be a cause of jaundice; gall stones could result from the finding that estrogens increase cholesterol concentrations in bile; estrogen-induced hypertension could be caused by increased hepatic synthesis of renin substrate. Many environmental agents possess direct estrogenic activity; i.e. they bind to estrogen receptors. Therefore, a critical need exists to determine if the cellular machinery required for estrogen action is present in the liver and to ascertain whether the biologic and/or toxic responses to estrogens in the liver is associated with specific forms of estrogen binding proteins. We have characterized specific hepatic cytosolic receptors for estrogens and demonstrated nuclear translocation of estrogen receptor complex in a cell free system using the rat as an experimental animal. Additionally, our studies are investigating sex differences in estrogen action and the similarities and differences of estrogen action in the liver compared to other target tissues such as the uterus.

The response of the liver to xenobiotics in some cases also appears to undergo sex-differentiation and this sex-differentiation is under pituitary-hypothalamic-gonadal regulation. Our findings also indicate that changes in the hormonal environment can qualitatively and irreversibly alter hepatic function and the hepatic response to xenobiotics. These studies provide insight into the mechanisms whereby changes in hormonal milieu might modify the response to toxicants including carcinogens.

PUBLICATIONS

Lucier, G. W., Davis, J. G., and McLachlan, J. A.: Transplacental toxicity of PCBs and PBBs: Methodological approach. 17th Hanford Biology Symposium - Developmental Toxicology of Energy-Related Pollutants. Technical Information Center, Department of Energy, 188-204, 1978.

Lucier, G. W.: Pharmacological and toxicological significance of UDP glucuronyltransferase during development in Conjugation Reactions in Drug Biotransformation, A. Aito (Ed) Elsevier Press, 167-178, 1978.

Lamartiniere, C. A. and Lucier, G. W.: Fetal programming of hepatic histidase by diethylstilbestrol. J. Steroid Biochem. 9, 1978.

Lucier, G. W., Schiller, C. A., McDaniel, O. S., and Matthews, H. B.: Structural requirements for the accumulation of PCB metabolites in the fetal intestine. Drug Metab. Dispos. 6, 584-590, 1978.

Schiller, C. M. and Lucier, G. W.: Differential susceptibility of intestinal crypt and tip cells to TCDD inductive actions. Chem. Biol. Interact. 22, 199-209, 1978.

Powell-Jones, W., Nayfeh, S. N., and Lucier, G. W.: Multiple forms of estrogen receptors in rat liver nuclei. Biochem. Biophys. Res. Commun. 85(1) 167-173, 1978.

and is associated with the amount of cadmium sensitive forms of cytochrome P-450 as demonstrated by synthesis/degradation studies on hepatic hemoproteins. The levels of cadmium sensitive forms appear to be imprinted during a critical period of neonatal development by androgens.

Administration of methadone to male neonates resulted in female type Km values for the hepatic monooxygenase system in the subsequent adult animal whereas V_{max} values were unchanged. This feminization of kinetic values appears to be related to a methadone-mediated depression of testosterone levels in neonates suggesting that methadone prevents the imprinting of the monooxygenase system. This idea was reinforced by the finding that simultaneous administration of testosterone and methadone to neonates resulted in normal sex differentiation of enzyme activity.

Recent evidence suggest that receptors in hepatic cytosol for polycyclic hydrocarbons are required for the induction of cytochrome P-448-catalyzed enzyme activities. It has been postulated that cytoplasmic receptor-ligand complex translocates to the nucleus in a manner analogous to that described for the steroid hormone receptor. To examine the mode of induction of hepatic enzyme activities by non-steroidal inducing agents, we have investigated the effect of hypophysectomy on the induction of hepatic monooxygenase activities by 3,4,3',4'-tetrachlorobiphenyl (4-CB) (3-MC type inducer) and 2,4,5,2',4',5'-hexachlorobiphenyl (6-CB) (phenobarbital-type inducer). Treatment of sham-hypophysectomized (Hx) rats with 4-CB (initiated at 12 days following surgery) resulted in a 30- and 3-fold increase in 7-ethoxyresorufin o-deethylation (ERD) activity and chtochrome P-448 contents, respectively. This inductive effect of 4-CB was reduced significantly in the absence of pituitary glands. Exposure of sham-Hx rats to 6-CB resulted in significant increases in hepatic ED activity and cytochrome P-450 levels; however, the magnitude of induction by 6-CB was not altered by prior-hypophysectomy (12 days). These data suggest that pituitary glands may play a role in the induction of cytochrome P-448-catalyzed reactions but not cytochrome P-450-dependent reactions. However, when the inductive effect of 4-CB was examined at 21 or 28 days following Hx, the extents of the induction of ERD activity and cytochrome P-448 contents were of the same order of magnitude as those detected in sham-Hx rats. The time-dependent restoration of normal hepatic responsiveness to 4-CB in Hx rats suggest development of compensatory or alternative mechanisms for cytochrome P-448 induction.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: During recent years it is becoming increasingly evident that the liver may be an toxicologically important target organ for estrogens. For example there are many adverse side effects of oral contraceptives that could be related to estrogen action in the liver. Some of these side effects are; increased incidence of 1) thrombosis, 2) heart attacks, 3) jaundice, 4) gall stones, 5) hypertension, and 6) benign hepatomas. The occurrence of thrombosis may be related to increased synthesis of plasma clotting factors; cardiovascular disease could be enhanced by increased hepatic synthesis of plasma triglycerides and certain lipoproteins; estrogen alters the hepatic transport of bile acids and bilirubin and therefore might be a cause of jaundice; gall stones could result from the finding that estrogens increase cholesterol concentrations in bile; estrogen-induced hypertension could be caused by increased hepatic synthesis of renin substrate. Many environmental agents possess direct estrogenic activity; i.e. they bind to estrogen receptors. Therefore, a critical need exists to determine if the cellular machinery required for estrogen action is present in the liver and to ascertain whether the biologic and/or toxic responses to estrogens in the liver is associated with specific forms of estrogen binding proteins. We have characterized specific hepatic cytosolic receptors for estrogens and demonstrated nuclear translocation of estrogen receptor complex in a cell free system using the rat as an experimental animal. Additionally, our studies are investigating sex differences in estrogen action and the similarities and differences of estrogen action in the liver compared to other target tissues such as the uterus.

The response of the liver to xenobiotics in some cases also appears to undergo sex-differentiation and this sex-differentiation is under pituitary-hypothalamic-gonadal regulation. Our findings also indicate that changes in the hormonal environment can qualitatively and irreversibly alter hepatic function and the hepatic response to xenobiotics. These studies provide insight into the mechanisms whereby changes in hormonal milieu might modify the response to toxicants including carcinogens.

PUBLICATIONS

Lucier, G. W., Davis, J. G., and McLachlan, J. A.: Transplacental toxicity of PCBs and PBBs: Methodological approach. 17th Hanford Biology Symposium - Developmental Toxicology of Energy-Related Pollutants. Technical Information Center, Department of Energy, 188-204, 1978.

Lucier, G. W.: Pharmacological and toxicological significance of UDP glucuronyltransferase during development in Conjugation Reactions in Drug Biotransformation, A. Aito (Ed) Elsevier Press, 167-178, 1978.

Lamartiniere, C. A. and Lucier, G. W.: Fetal programming of hepatic histidase by diethylstilbestrol. J. Steroid Biochem. 9, 1978.

Lucier, G. W., Schiller, C. A., McDaniel, O. S., and Matthews, H. B.: Structural requirements for the accumulation of PCB metabolites in the fetal intestine. Drug Metab. Dispos. 6, 584-590, 1978.

Schiller, C. M. and Lucier, G. W.: Differential susceptibility of intestinal crypt and tip cells to TCDD inductive actions. Chem. Biol. Interact. 22, 199-209, 1978.

Powell-Jones, W., Nayfeh, S. N., and Lucier, G. W.: Multiple forms of estrogen receptors in rat liver nuclei. Biochem. Biophys. Res. Commun. 85(1) 167-173, 1978.

Lucier, G. W. and McDaniel, O. S.: Developmental toxicology of the organohalogens, effects on enzyme development. Ann. N. Y. Acad. Sci. (in press, 1979).

Lucier, G. W., Lamartiniere, C. A., Dieringer, C. A., and Lui, E.: Metabolic activation/deactivation during development. Environ. Hlth. Perspec. 29: 17-26, 1979.

Lamartiniere, C. A., Dieringer, C. S. and Lucier, G. W.: Altered sexual differentiation of hepatic UDP glucuronyltransferase by neonatal hormone treatment. Biochem. J. 180, 313-318, 1979.

Tilson, H. A., Davis, G. J., McLachlan, J. A., and Lucier, G. W.: Behavioral changes in mice by prenatal PCB exposure. Environ. Res. (in press).

Dieringer, C. S., Lamartiniere, C. A., Schiller, C. M. and Lucier, G. W.: Altered sexual differentiation of hepatic steroid-metabolizing enzymes by neonatal exposure to PCB congeners in rats. Biochem. Pharmacol. (in press).

Dieringer, C. S., Lamartiniere, C. A. and Lucier, G. W.: Sex differences in pituitary-gonadal regulation of 5α-reductase and 16α-hydroxylase. J. Ster. Biochem. (in press).

Lucier, G. W. and McDaniel, O. S.: Pituitary-gonadal regulation of testosterone glucuronidation in rat liver. J. Appl. Biochem. (in press).

Lamartiniere, C. A., Dieringer, C. S. and Lucier, G. W.: Altered ontogeny of glutathione-S-transferase by 2,4,5-2',4'5'-hexachlorobiphenyl in rat liver. Toxicol. Appl. Pharmacol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70132-01 LET

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Gastrointestinal Function and Toxicology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. M. Schiller	Head, Gastrointestinal	LET/LOFT	NIEHS
OTHER:	G. W. Lucier	Acting Lab Chief	LET/LOFT	NIEHS
	B. A. Fowler	Head, Renal and Intracellular	LET/LOFT	NIEHS
	F. Talley	Biologist	EBCB	NIEHS

COOPERATING UNITS (if any)
Environmental Biology and Chemistry Branch, NIEHS
Dept of Med., Chapel Hill
Research Triangle Institute
Dept. Biochem. & Nutrition, Chapel Hill

LAB/BRANCH
Laboratory of Environmental Toxicology/Laboratory of Organ Function Toxicology

SECTION
Gastrointestinal

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 3.1	PROFESSIONAL: 1.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long-range purpose of this project to study the development of the functions of the small intestine in normal and environmentally, nutritionally and hormonally-stressed animals. The current functional and toxicological studies that relate to developing and adult animals are: (1) ontogeny of intestinal enzymes and isozymes involved in energy metabolism, i.e., lactate dehydrogenase, NAD-and Fp-glycerol 3-phosphate dehydrogenases, glutamine metabolizing enzymes, gluconeogenic enzymes, and β-glucuronidase, (2) characterization of the morphology and metabolism of natural and foreign substances in isolated intestinal absorptive cells, and (3) monitoring the response of key intestinal enzymes to in vivo treatments with colon-specific carcinogens, polyhalogenated aromatics and heavy metals.

PROJECT DESCRIPTION

METHODS EMPLOYED: The preparation of the different subcellular fractions from the various intestinal tissues as well as the numerous metabolic and enzyme assays have been described in detail before and are contained in the bibliographic references. Of special interest is the method we devised for the isolation of intestinal cells that yields monodispersed suspensions.

Isolation of Intestinal Epithelial Cells. A 20-cm segment of the intestine immediately distal to the pylorus was excised and perfused with cold saline solution. The protease/EDTA method requires that the everted intestinal segments be filled to slight distention and the villous cells were harvested by incubation in a protease (10 U/ml)/EDTA (1 mM) in medium. The medium was Hank's balanced salt solution (Mg, Ca and glucose-free) which contained bovine serum albumin (0.5%, w/v) and Tris-HCl (20 mM), pH 7.4. The intestinal segments were incubated with protease/EDTA in medium for 10 min followed by incubation with medium only for 15 min. The eversion of the intestinal segment while exposed to the protease/EDTA medium allowed for better control of the pH of the medium. The released cells were washed twice in medium by centrifugation at 150 x g for 1 min at 4°C and resuspending in fresh medium. The protease/EDTA released cells were examined for cell permeability based on the exclusion of a solution of Trypan blue dye (0.04%, w/v). Comparisons of metabolic activities between this method and the mechanical/chelation method of cell preparation were made on the basis of milligram of cell protein as the intestinal cells obtained by the vibration method tend to aggregate which made accurate counts of cell numbers difficult (Schiller and Lucier, 1978).

MAJOR FINDINGS AND PROPOSED COURSE: Lactate dehydrogenase. Our study of the developing small intestine presents a detailed report of the catalytic, isozymic and kinetic properties of lactate dehydrogenase (LDH). These results indicate that although there was a marked increase in LDH activity in the forward and reverse directions during the postnatal period, the activity of the reverse reactions (pyruvate as substrate) was 10-fold higher than the forward reaction (lactate as substrate) throughout development. The isozymic and catalytic properties in the presence of NHXDH suggest similarities between the intestinal and leg muscle form of LDH (M_4). The Km (pyruvate) is considerably lower than the Km (lactate) throughout development and is close to the tissue value of pyruvate previously reported (Schiller, 1977). These results allow for a better understanding of the properties of this enzyme during development as well as relating these properties to the changing metabolic role of this tissue during development.

Isolated Cells. We have observed that isolated intestinal cells can be prepared by the protease/EDTA method so that uniformly monodispersed cell suspensions are produced. These cell suspensions repeatedly excluded trypan blue dye at a level of 90-95%. Microscopic examination of these cells revealed structurally intact surface membranes and subcellular organelles. Unlike the mechanical/chelation method which yields clumps of columnar cells, these cells are monodispersed spherical cells. Transmission and scanning electron photomicrographs illustrate the complexity and degree of interdigitation between cells that provide the structural rigidity of the intestinal epithelial wall. The metabolic activity of the monodispersed cells was high and sustained as indicated by oxygen consumption and by carbon dioxide production over a period of one hour. These cells preferentially and more rapidly utilize L-glutamine compared to β-D-glucose. We are currently examining the pathway of glutamine utilization by intestinal cells. We suggest that the rapid rate of oxidation of L-[U-^{14}C]-glutamine to CO_2 which is not diluted by the presence of equimolar β-D [U-^{12}C]-glucose is indicative of a tissue that rapidly generates its own acetyl-CoA by the P-enolpyruvate intermediate.

Other uses of the isolated cell techniques have been the evaluation of the cell-specific induction of uridine-3'-5'-diphosphate (UDP)-glucuronyltransferase by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure and of morphological properties of these cells. We propose to utilize the isolated cell techniques to examine other specific proteins associated with metal absorption and ageing e.g. metallothionein; Ca transport, cell-specific regulatory proteins.

Metabolic Studies. Earlier studies from our laboratory revealed that prolonged oral exposure to arsenic specifically interfered with the normal rates of mitochondrial pyruvate utilization (Fowler et al., 1977 and Schiller et al., 1977). The inhibition of pyruvate dehydrogenase activity both before and after Mg-activation suggests an arsenic effect on mitochondrial pyruvate metabolism which, in part, involves inhibition of pyruvate decarboxylase. Evidence is also presented which may indicate an arsenic effect on the kinase and/or phosphatase which regulates pyruvate dehydrogenase activity. More recently, we have been interested in the possible interaction between prolonged arsenic exposure and the diabetic state. We have examined both hepatic lipogenesis and renal integrity in control, arsenic, alloxan-diabetic and arsenic alloxan-diabetic rats. The results from the urinary enzyme measurements from the animals exposed to 0 and 40-ppm arsenic for three weeks and/or to alloxan-induced diabetes support several statistically significant ($P<0.10$) observations: (1) lactate dehydrogenase, L-glutamate oxalo-acetate transaminase, acid phosphatase, β-glucuronidase and N-acetyl-β-D-glucosaminidase are elevated in the alloxan-diabetic arsenic rats as compared to either controls; (2) alkaline phosphatase is elevated in the normal arsenic rats as compared to the normal controls; and (3) acid phosphatase is elevated in the alloxan-diabetic control rats as compared to normal control rats. We are attempting to repeat this experimental approach by inducing the diabetic state with alloxan first and then exposing them to arsenic while receiving insulin therapy.

Colon-specific Carcinogenesis. Our studies on the tissue specificity of the effects of the colon-specific carcinogen: 1,2-dimethylhydrazine (DMH) have focused on intestinal β-glucuronidase and dehydrogenases. β-glucuronidase isozymic profiles were examined to evaluate the possible utility of this enzyme as an early indicator of toxic response. The intestinal β-glucuronidase activity may also play an important role in the enterohepatic circulation of active DMH metabolites. In addition, our studies of the L-glycerol 3-phosphate dehydrogenases evaluate the possible role of the L-glycerol 3-phosphate substrate cycle in the regulation of colonic and tumor energy metabolism. These colonic dehydrogenases may also be involved in the oxidation of a DMH metabolite to form an active aldehyde.

Proposed Course. We plan to concentrate our efforts in fiscal year 1980 on follow-up studies based on some of our current projects. In particular, we are interested in studying (1) the metabolic pathway for the preferential utilization of L-glutamine by intestinal cells; (2) the effects of prenatal and postnatal exposure to 1,2-dimethylhydrazine on NAD-and Fp-L-glycerol 3-phosphate dehydrogenases and other metabolic energy related enzymes in the colonic tissues; (3) the effects of prenatal exposure to environmental compounds on key intestinal transport and absorptive functions; and (4) the effects of altered redox state on the transport and metabolism of p-nitrophenol analogue by the isolated intestinal cells.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In the past, perinatal development studies of the small intestine have dealt primarily with hydrolytic enzymes, the brush border, and the absorption of specific nutrients. Our emphasis has been on the development of the metabolic energy producing processes required for the active transport of nutrients and also the effect of various bioactive environmental compounds on the normal developmental patterns of this target organ. Currently, our laboratory is using a variety of biochemical and morphological techniques to study several aspects of the development of normal intestinal function, mechanisms of intestinal toxicity and indicators for response to toxic insults.

Many of our results have long-range significance in providing a better understanding of the development of normal intestinal function, the normal morphology and metabolism of intestinal cells and the unique mitochondrial and energy metabolism of intestinal tissue. Other observations include results which may have toxicological and clinical utility for the medical community. Examples of the latter include our studies on the responses to treatments with 1,2-dimethylhydrazine, 2,3,7,8-tetrachlorodibenzo-p-dioxin and arsenic.

PUBLICATIONS

Schiller, C. M., Fowler, B. A. and Woods, J. S.: Pyruvate metabolism after *in vivo* exposure to oral arsenic. *Chem. Biol. Interactions* 22: 25-33, 1978.

Schiller, C. M. and Lucier, G. W.: The differential response of isolated intestinal crypt and tip cells to the inductive actions of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Chem. Biol. Interactions* 22: 199-209, 1978.

Lucier, G. W., McDaniel, O. S., Schiller, C. M. and Matthews, H. B: Structural requirements for the accumulation of PCB metabolites in the fetal rat intestine. *Drug Metab. Dis.* 6: 584-590, 1978.

Schiller, C. M.: Flow of reducing equivalents into isolated intestinal mitochondria. Metabolism 28: 105-112, 1979.

Schiller, C. M., Walden, R., Kee, T. E.: Effects of hydrazine and its derivatives on the development of intestinal brush border enzymes. Toxicol. Appl. Pharmacol. (in press).

Dieringer, C. S., Lamartiniere, C. A., Schiller, C. M. and Lucier, G. W.: Structure-activity relationships for altered ontogeny of hepatic steroid-metabolizing enzymes by PCB congeners. Biochem. Pharmacol. (in press)

Fowler, B. A., Woods, J. S. and Schiller, C. M.: Studies of hepatic mitochondrial structure and function I. Morphometric and biomedical evaluation of *in vivo* perturbation by arsenate. Lab. Invest. (in press)

Schiller, C. M. and Walden, R.: Processes of transport and absorption in the developing infant intestine. Rev. of Infec. Dis. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70135-03 LET

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Effect of Hormones and Xenobiotics on the Regulation of Perinatal Enzyme Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. A. Lamartiniere	Senior Staff Fellow	LET/LOFT	NIEHS
OTHER:	G. W. Lucier	Acting Lab Chief	LET/LOFT	NIEHS
	W. Powell-Jones	Visiting Fellow	LET/LOFT	NIEHS
	N. Illsley	Visiting Fellow	LET/LOFT	NIEHS
	E. Lui	Visiting Fellow	LET/LOFT	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Toxicology/Laboratory of Organ Function and Toxicology

SECTION
Hepatic and Endocrine

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 3.5	PROFESSIONAL: 1.0	OTHER: 1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our research efforts are directed toward investigating the effect and mode of action of hormones and xenobiotics on the regulation of enzyme patterns during perinatal development. Particular emphasis has been placed on the postnatal development of hepatic enyzmes in the rat following perinatal exposure to gonadal hormones [testosterone propionate (TP) or estradial-17-β(E_2)] and hormonally active xenobiotics [polychlorinated biphenyl (PCB), diethylstilbestrol (DES), dichloro-diphenyl-trichloro-ethane (DDT) and methoxychlor]. We have investigated the altered ontogeny and sex differentiation of several hepatic enzyme markers following prenatal exposure to the above mentioned chemical and the results of our studies on the role of the hypothalamic-pituitary-gonadal axis on the regulation of some of these enzymes. These studies have demonstrated that the regulation of hepatic enzyme development is different for different groups of enzymes and that the critical period for neonatal imprinting of enzyme activity is not the same for all enzymes. Further studies are investigating altered sex differentiation of hepatic enzymes by mapping protein profiles on 2-D gels.

PROJECT DESCRIPTION

METHODS EMPLOYED: Neonatal exposure to TP, E_2 or DES is carried out by subcutaneous injections of 1.45 µmoles of the hormone or 0.02 ml propylene glycol (PG) to Sprague-Dawley CD rats on days 2,4,6 post-partum. DDT or methylchor is administered under the same schedule at concentrations of 1 mg or 3 mg/animal. The PCBs were administered orally using corn oil as a vehicle on alternate days from 8-18th day of gestation. Transplantation of pituitaries were done by placing pituitaries of age-matched rats under the kidney capsule of hypophysectomized rats anesthesized with nembutal. Gonadectomies and hypophysectomies were performed at different developmental stages.

MAJOR FINDINGS AND PROPOSED COURSE: Visual observations of postnatally programmed rats revealed that testes of normal males were descended at 21 days but those of TP and DES animals decended later (35 and 63 days respectively). These TP and DES treated animals had smaller testes at prepubertal and adult ages. Vaginal openings in normal females occurred around 42 days of age but vaginal openings in TP and DES treated animals did not occur through 63 days of age. While control and TP females had similar uterine wet weights at 21 days of age, DES females had heavier uteri (may be due to higher circulating estrogens from DES treatment). In contrast adult DES and TP females had uterine weights lower than that of controls. An attempt to produce a uterotrophic response in 21 day old females was positive in control- and TP- females, but no elevation in uterine wet weights occurred in DES females. However, exogenous E_2 administration to adult females, did elicit a uterotrophic response in control- and TP- and DES- animals.

The various enzyme markers that we have investigated as indicators of altered ontogeny of hepatic metabolism are histidase, 5α-reductase, UDP glucuronyltransferase, 16α-hydroxylase, ethylmorphine demethylase, aminopyrine demethylase, monoanimine oxidase and glutathione S-transferase. Neonatal administration of TP and DES did not have a significant effect on these enzymes at 21 days of age but alterations were observed in some of these enzyme markers at 63 days of age. Histidase activity, which is normally higher in females than males, was masculinized (decreased) by DES in adult females. Adult male glucuronyltransferase, 16α-hydroxylase, ethylmorphine demethylase, and aminopyrine demethylase (activities of these enzymes are normally higher in adult males than females) were feminized by neonatal TP and DES. Since no effect is seen in young animals and effects are seen in adult animals, TP and DES appear to be capable of altering normal enzyme programming by feminizing hepatic UDPGT, 16α-hydroxylase, ethylmorphine demethylase and aminopyrine demethylase in adult males. DES treatment of newborns masculinized histidase in the adult female. 5α- reductase and Glutathione S-transferase activities were unchanged at all ages tested.

Neonatal exposure of rats to DDT results in positive modulation of hepatic glutathione S-transferase, cytochrome P-450 content, and UDP glucuronyltransferase in 21 day old animals but little or no effect in the resulting 63 day old animals. Hepatic monoamine oxidase activity levels are unchanged at 21 days of age but at 63 days of age activity levels are elevated (feminization). Methoxychlor had no effect at all ages investigated.

Our experiments on the gestational exposure of rats to PCBs demonstrate that 3,3',4,4'-tetrachlorobiphenyl (4-CB) resulted in lower histidase levels in 6 day old offsprings. Activity levels were, however, back to normal in 20 day old animals. While glutathione S-transferase was unaffected by the 4-CB, positive modulation was observed in 6 to 55 day old offspring of 2,2',4,4',5, 5'-hexachlorobiphenyl (6-CB) exposed (gestational) rats. These results indicate that hormonally active xenobiotics administered during gestation are capable of altering the normal sequence of postnatal development.

In an attempt to understand the mechanism of postnatal programming of hepatic enzymes, we have investigated the role of the hypothalamic-pituitary-gonadal axis. Gonadectomy of normal rats revealed that high female histidase levels are dependent on the ovaries and that estrogen is responsible for these higher levels in the adult females. Hypophysectomy of males results in a dramatic increase in activity. However; upon transplantation of a pituitary under the kidney capsule of hypophysectomized male or female rats, no significant effect was noted. Likewise subcutaneous injections of FSH and LH to hypophysectomized rats had no effect. Castration of male or female rats had no significant effect on adult glutathione S-transferase enzyme activities but hypophysectomy resulted in significantly elevated enzyme levels in both sexes. Pituitary transplants in these hypophysectomized animals decreased glutathione S-transferase activity to levels intermediate to those of hypophysectomized and normal animals. Subcutaneous injections of FSH and LH to hypophysectomized male and female rats had a similar effect as the ectopic pituitary. These experiments suggest the involvement of the hypothalamic-pituitary-gonadal axis in the regulation of hepatic histidase and glutathione S-transferase.

We are currently expanding these investigations to the measurement of circulating gonadotrophins, pituitary hormones and specific receptors and correlating behavioral changes between control and treated animals. We are also evaluating hepatic protein profiles of normal and programmed animals by two-dimensional gel electrophoresis. Since a pituitary requirement is necessary for complete and normal expression of the developmental pattern of these hepatic enzymes, we are initiating in vitro studies to complement our present ongoing in vivo investigations. Using an established hepatoma cell line, we are studying the effect of hormones, pituitary extracts and hormonally active xenobiotics on metabolism. This in vitro model will enable us to better elucidate the role of the pituitary in the regulation of hepatic metabolism and the mechanism whereby chemicals can alter this process.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: One of the dominant biochemical aspects of the orderly development of an organism from fertilization to maturity is the continuous progression of enzyme protein formation ultimately resulting in specific enzymatic patterns which constitute and determine the metabolic machinery characteristic of each mature differentiated tissue. The postnatal development of sex-dependent hepatic mammalian enzymes is an excellent system to investigate ontogeny. It is the purpose of our studies to further our understanding of the factors responsible for complex postnatal developmental patterns. In addition to studying the normal expression of these enzymes we want to see if xenobiotics can qualitatively and/or quantitatively alter the normal expression of enzyme development, and discover, if so, how this is accomplished. The fetus and newborn are exposed to numerous factors that can have deleterious effects on the developing offspring. The studies reported above are examples of alterations of enzyme expression that may be markers for abnormal differentiation and development. These effects on enzyme development may result in an altered capacity to metabolize potential teratogens, carcinogens, or drugs and, therefore, allow humans to be more susceptible to biochemical insult. An understanding of these biochemical mechanisms should allow us to make predictions of chemicals that might alter normal biochemical functions of the liver.

PUBLICATIONS

Lamartiniere, C. A. and Lucier, G. W.: Programming of hepatic histidase following prenatal administration of diethylstilbestrol. J. Steroid Biochem. 9, 595-598, 1978.

Lamartiniere, C. A., Dieringer, C. S., Kita, E. and Lucier, G. W.: Altered sexual differentiation of hepatic UDP glucuronyltransferase by neonatal hormone treatment in rats. Biochem. J. 180, 313-318, 1979.

Illsley, N. P. and Lamartiniere, C. A.: Prenatal programming of hepatic monoamine oxidase by 5,5-diphenyl hydantion. Biochem. Pharmacology. (in press).

Lucier, G. W., Lui, E. M. K. and Lamartiniere, C. A.: Metabolic activation/deactivation reactions during prenatal development. Environ. Hlth. Perspectives (in press).

Dieringer, C. S., Lamartiniere, C. A. and Lucier, G. W.: Sex differences in pituitary-gonadal regulation of hepatic 5α-reductase and 16α-hydroxylase. J. Steroid Biochem. (in press).

Dieringer, C. S., Lamartiniere, C. A., Schiller, C. M. and Lucier, G. W.: Altered ontogeny of hepatic steroid-metabolizing enzymes by-pure PCB filler cogeners. Biochemical Pharmacology (in press).

Lamartiniere, C. A., Dieringer, C. S. and Lucier, G. W.: Altered ontogeny of glutathione S-transferase 2,4,5-2',4',5'-hexochlorobiphenyl. Toxicol. Appl. Pharmacol. (in press).

Lamartiniere, C. A.: Neonatal estrogen treatment alters sexual differentiation of hepatic histidase. Endocrinology (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70200-06 LET

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Morphologic and Biochemical Effects of Toxic Metals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. A. Fowler	Head, Renal Intracellular Toxicology	LET/LOFT	NIEHS
OTHER:	C. M. Schiller	Research Chemist	LET/LOFT	NIEHS
	N. G. Carmichael	Visiting Fellow	LET/LOFT	NIEHS
	K. S. Squibb	Postdoctoral Fellow	LET/LOFT	NIEHS
	R. D. Kardish	Visiting Fellow	LET/LOFT	NIEHS

COOPERATING UNITS (if any)

Department of Pathology, UNC
FDA, Cincinnati, Ohio
Department of Biochemistry, Duke
Department of Environmental Hygiene, Karolinska Institute

LAB/BRANCH

~~Laboratory of Environmental Toxicology/Laboratory of Organ Function Toxicology~~

SECTION

Renal and Intracellular

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.6	4.6	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

When animals are exposed to different trace metals for prolonged time periods, each metal produces a biological response profile which specifically characterizes exposure to that metal. The objective of these studies is to assess and characterize response profiles based on a thorough understanding of subcellular mechanisms of metal toxicity and specifically to (1) define and correlate ultrastructural and biochemical responses in vivo which characterize exposure to toxic trace elements following prolonged exposure and (2) develop early, specific, and sensitive, biochemical testing procedures that may be used to evaluate human populations exposed to environmentally important trace elements. Specific metals and areas of interest include the biochemical effects of cadmium, manganese, arsenic, mercury, selenium, indium and lead on mitochondrial, microsomal and lysosomal structure and function: Enzyme activities associated with mitochondrial membranes have been found to show early and pronounced changes in mitochondria of rats and mice following prolonged exposure to arsenic and methyl mercury. Further development of these biochemical response profiles should permit a more basic understanding of the cellular mechanisms of metal toxicity and development of metal-specific biochemical testing procedures which accurately assess a biological response to these agents prior to overt toxicity.

PROJECT DESCRIPTION

METHODS EMPLOYED: Quantitative transmission electron microscopy; X-ray microanalysis; biochemical assays of mitochondrial respiration; and lysosomal, microsomal, cytosolic, and mitochondrial enzymes. Sephadex and DEAE chromatography, electrophoresis, isolectric focusing and amino acid analysis of metal binding proteins from mammals and marine shellfish. Tissue analysis for metals by atomic absorption spectroscopy, plasma emission spectroscopy, and proton induced X-ray emission analysis.

MAJOR FINDINGS AND PROPOSED COURSE: (1) Lead x Cadmium x Arsenic Interaction Rats were exposed to dietary lead (200 ppm), cadmium (50 ppm) or arsenic (50 ppm) as arsenate (InAs) or arsanilic acid (OAs) either alone or in combination for 10 weeks using 2x2x3 factorial design. Administration of Pb produced the distinctive ultrastructural changes characterized by swollen proximal tubule cell micochondria and intranuclear inclusion bodies which were shown by X-ray microanalysis to contain the highest measurable intracellular concentrations of Pb. These findings were observed in all Pb treatment groups except those receiving concommitant Cd exposure which showed decreased numbers of inclusions and a 60% reduction in total mean renal lead values measured by plasma emission spectroscopy. Cd exposure also reduced renal concentrations of Fe by 30% and increased that of Zn by 20% but did not influence renal concentrations of As, Cu, Ca, Mg, Mn or P. Exposure to InAs but not OAs produced mild swelling of tubule cell mitochondria and increased mean total renal Cu to 200% of control. These studies indicate that interactions between common toxic elements do occur and are characterized by alterations which influence renal trace metal levels. Previous studies on the effects of these metals individually have shown both lead and arsenic to produce specific urinary porphyrin excretion patterns dominated by uroporphyrin and coproporphyrin. These metal-specific patterns were found to be altered by combined Pb x As treatments and thus indicating that these biological indicators of toxicity are changed in multi-element exposure situations.

(2) Cadmium Metellothionein Toxicity Circulating cadmium-thionein (Cd-MT) is cleared from the mammalian circulatory system by filtration through the kidney glomerulus with subsequent reabsorption by kidney proximal tubules. Damage to the tubules results following uptake of Cd-MT, which is dependent upon time and the dose levels of cadmium administered. Intravenous administration of ^{109}Cd-MT at doses of 0.017 and 0.17 mg Cd/kg body weight with examination of total renal uptake of ^{109}Cd at 0.5, 3, and 24 hr disclosed that the rate of clearance from the blood and uptake by the kidney was significantly more rapid at the 0.017 mg Cd/kg dose. Ultrastructural changes resulting from intravenous injection of either form A or B of Cd-MT were characterized by increased numbers of pinocytotic vesicles and small, dense lysosomal structures. There was no evidence of mitochondrial swelling or cell death at either 3 or 6 hr after injection. The subcellular distribution of cadmium in kidney tissue at various times after administration of Cd-MT was determined by using differential centrifugation techniques with ^{109}Cd and in situ by using x-ray microanalysis. At 30 min after injection of Cd-MT, significant amounts of cadmium were present in lysosomal

fractions indicating an interaction between the tubular lysosome system and Cd-MT prior to the onset of overt cellular toxicity. Results suggest that Cd-MT is reabsorbed and broken down by kidney tubule cells in a physiological manner with possible subsequent release of the toxic cadmium ion.

Trace Metal Accumulation in Shellfish Kidneys X-ray microanalysis studies have shown that crystalline concretions in kidneys of marine shellfish contain high levels of many metals such as calcium, manganese, zinc and iron. Studies on several species of scallops have shown considerable inter-animal and inter-species variation in there elements. Studies with scallops using ^{54}Mn, ^{65}Zn, and ^{109}Cd showed major differences in the intracellular compartmentation of these metals with 34.3% of the ^{54}Mn, 5.6% of the ^{65}Zn, and 1.3% of the ^{109}Cd in concretions following short-term exposure. Extensive binding of these elements to soluble low molecular weight metal-binding proteins was also observed indicating that several compartments may be involved in the renal metabolism of these metals by shellfish.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies attempt to characterize and delineate the subcellular mechanisms of trace metal toxicity following prolonged exposure by combined ultrastructural, and biochemical techniques. Once sufficient knowledge in this area is obtained, it may be applied to the development of metal-specific biochemical testing procedures which will accurately reflect a preclinical biological response to toxic trace metal exposure in human populations.

PUBLICATIONS

Schiller, C. M., Fowler, B. A., Woods, J. S.: Pyruvate metabolism after *in vivo* exposure to oral arsenic. *Chem. Biol. Interactions* 22: 52-33, July 1978.

Fowler, B. A. and Mahaffey, K. R.: Interactions between lead, cadmium, and arsenic in relation to porphyrin excretion patterns. *Environ. Hlth. Perspec.* 25: 87-90, August 1978.

Fowler, B. A. and Nordberg, G. F.: The renal toxicity of cadmium metallothioein: Morphometric and X-ray analytical studies. *Toxicol. Appl. Pharmacol.* 46: 609-624, December 1978.

Fowler, B. A. and Nordberg, G. F.: The renal toxicity of cadmium metallothionein: Morphometric and X-ray microanalytical studies. *Toxicol. Appl. Pharmacol.* 46: 609-624, December 1978.

Squibb, K. S., Ridlington, J. W., Carmichael, N. G. and Fowler, B. A.: Early cellular effects of circulating cadmium-thionein on kidney proximal tubules. *Environ. Hlth. Perspec.* 28: 301-304, February 1979.

Fowler, B. A.: The international conference on environmental cadmium: An overview. *Environ. Hlth. Perspec.* 28: 287-196, February 1979.

Ridlington, J. W. and Fowler, B. A.: Isolation and partial characterization of a cadmium-binding protein from the American oyster (Crassostrea virginica). Chem. Biol. Interact. (in press).

Fowler, B. A. and Woods, J. S.: The effects of prolonged oral arsenate exposure on liver mitochondria of mice: Morphometric and biochemical studies. Toxicol. Appl. Pharmacol. (in press).

Woods, J. S., Carver, G. T. and Fowler, B. A.: Altered regulation of hepatic heme metabolism by indium chloride. Toxicol. Appl. Pharmacol. (in press).

Carmichael, N. G. and Fowler, B. A.: Effects of separate and combined chronic mercuric chloride and sodium selenate administration in rats: Histological, ultrastructural and X-ray microanalytical studies of liver and kidney. J. Env. Pathol. Toxicol. (in press).

Carmichael, N. G., Squibb, K. S. and Fowler, B. A.: Metals in the molluscan kidney: A comparison of two closely related species (Argopecten) using X-ray microanalysis and atomic absorption spectroscopy. J. Fish. Res. Bd. Canada. (in press).

Fowler, B. A., Woods, J. S. and Schiller, C. M.: Studies of hepatic mitochondrial structure and function I. Morphometric and biochemical evaluation of in vivo perturbation by arsenate. Lab. Invest. (in press).

Fowler, B. A. and Nordberg, G.: Silver. In Friberg, L., Nordberg, G. F., and Vouk, V. (eds): Handbook on the Toxicology of Metals. Elsevier Pub. Co. (in press).

Fowler, B. A. and Vouk, V.: Bismuth. In Friberg, L., Nordberg, G. F., and Vouk, V. (eds.): Handbook of the Toxicology of Metals. Elsevier Pub. Co. (in press).

Fowler, B. A.: Indium. In Friberg, L., Nordberg, G. F., and Vouk, V. (eds.): Handbook on the Toxicology of Metals. Elsevier Pub. Co. (in press).

Tsuchiya, K. Fowler, B. A., Ishinishi, N., and Vahter, M.: Arsenic. In Friberg, L., Nordberg, G. F., and Vouk, V. (eds.): Handbook on the Toxicology of Metals. Elsevier Pub. Co. (in press).

Carmichael, N. G., Squibb, K. S., Engel, D. W. and Fowler, B. A.: Metals in the molluscan kidney: Uptake and subcellular distribution of ^{109}Cd, ^{54}Mn and ^{65}Zn by the clam Mercenaria mercenaria. Comp. Biochem. Biophys. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70210-02 LET

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of TCDD on the Disposition of Heme and Heme-Containing Proteins in Mammalian Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. T. Kitchin	Staff Fellow	LET/LOFT NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Environmental Toxicology/Laboratory of Organ Function Toxicology

SECTION
Hepatic and Endocrine

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies are designed to investigate the enhanced rate of benzo(α)pyrene metabolism following interaction of cytochrome P-448 obtained from TCDD treated rats with hepatic and renal microsomes low in endogenous cytochrome P-450 and MFO enzyme activity. TCDD is known to produce teratogenesis and fetal lethality in mammals as well as porphyria in a variety of species. TCDD also increases the activation of various exogenous carcinogenic hydrocarbons and alters the metabolism of endogenous steroid hormones in mammalian tissues. The present studies demonstrate that aryl hydrocarbon hydroxylase activity was increased 12, 26, 31 and 53 fold when 1.0 nmole of partially purified cytochrome P-448 was incubated with fetal liver microsomes, microsomes from kidney cortex of female rats and cumene hydroperoxide pretreated hepatic microsomes from female and male rats, respectively. CHP pretreatment increased both the absolute magnitude of and the relative increase in enhanced AHH rate in adult rat hepatic microsomes incubated with cytochrome P-448, while CHP pretreatment decreased these parameters in fetal hepatic and adult renal microsomes. Incubation with 1 nmole of exogenous cytochrome P-448 increased the AHH activity of NADPH-treated, lipid peroxidized microsomes by 76 fold, a value 20% higher than control microsomal AHH activity.

PROJECT DESCRIPTION

METHODS EMPLOYED: Microsomes were isolated by subcellular fractionation from liver and kidneys of adult male, adult female and 21 day old fetal rats. Some microsomal suspensions were incubated for 20 minutes at 37°C with either 2.0 mM cumene hydroxide or 1.25 mM NADPH, cooled, recentrifuged and resuspended before use. Hepatic microsomes from immature male rats given 10 μg/kg of TCDD orally were the source of cytochrome P-448 preparation. Cytochrome P-448 was solubilized and partially purified by ammonium sulfate precipitation, DEAE column chromatography, and calcium phosphate gel absorption and elution by the method of Lu and Levin. Solubilized cytochrome P-448 (1-4 nmol) and various microsomes (0.25-0.60 mg protein) were incubated together at 37°C for 30 min and then the AHH activity determined by either a fluorometric method using 3-hydroxybenzo(α)pyrene as an internal standard or by a radiometric method using 6-^{3}H-benzo(α)pyrene as the substrate. Statistical levels of significance were determined by using the two sided Student's t-test.

MAJOR FINDINGS AND PROPOSED COURSE: Completed in vivo studies indicate that TCDD (2 μg/kg) does not directly affect hepatic heme biosynthesis and does not increase the activity of δ-aminolevulinic acid synthetase, the rate limiting enzyme in that process, at any of the time points examined. However, a 45 fold increase in the activity of the cytochrome P-448-mediated aryl hydrocarbon hydroxylase was observed seven days after TCDD administration. TCDD induces the synthesis of apocytochrome P-448 which combines with heme to form new cytochrome P-448 and increased AHH activity. In vitro interaction studies using partially purified cytochrome P-448 from TCDD-treated rats and various microsomes low in cytochrome P-450 and MFO activity because of organ, sex and ontogenic reasons demonstrated enhanced rates of AHH activity (up to 76 fold). The increases in AHH activity observed when 0.25 to 1.0 nmol of cytochrome P-448 was incubated with hepatic microsomes from female rats was linear with respect to cytochrome P-448. The 6.3 and 4.3 fold increases obtained with the radiometric and fluorometric assays of AHH activity suggest that incubation of female hepatic microsomes with exogenous cytochrome P-448 increases the formation of the non-phenolic metabolites of benzo(α)pyrene to a greater extent than it does the fluorescent phenolic metabolites. Either organ, sex and ontogenic differences in microsomal AHH activity or cumene hydroperoxide pretreatment permit a more sensitive demonstration of the incorporation of enzymatically active exogenous cytochrome P-448 than is possible using hepatic microsomes obtained from adult male rats. Enzymatic interaction studies using control or chemically modified microsomes and exogenous cytochromes of the P-450 class may be useful in providing information about the characteristics of different microsomal membranes such as their ability to incorporate exogenous cytochromes and the efficiency with which these incorporated cytochromes couple with endogenous electron transport proteins

Further studies will determine if heme or the central metal ion iron is the endogenous repressor of δ-aminolevulinic acid synthetase and the inducer of heme oxygenase in mammalian liver. The effects of in vivo administration of protoporphyrin IX, heme, iron, bilirubin and EDTA on ALA synthetase and heme oxygenase activities as well as the *in vitro* inhibition ALA synthetase by various tetrapyroles will be determined. In a collaborative project with Dr. Sanyal of LET, metabolically active microsomes will be added to an *in vitro* embryo culture system to activate chemicals to their teratogenic metabolites. The growth and differentiation of day 11 rat embryos grown *in vitro* with culture media containing adult rat hepatic microsomes, NADPH and cyclophosphamide will be determined.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Although chronic exposure of rats to TCDD is known to increase the number of tumors of the respiratory system and liver while decreasing the number of tumors found in endocrine organs such as pituitary, uterus, mammary gland and adrenal glands, the effect of TCDD on human oncogenesis is unknown. TCDD produces teratogenesis and fetal lethality in experimental animals and is suspected of causing miscarriages in women environmentally exposed to high levels of TCDD. The completed *in vivo* and *in vitro* studies show TCDD greatly increases hepatic microsomal oxidative metabolism of benzo(a)pyrene, particularly in female rat liver. AHH activity was increased 12, 26, 31 and 53 fold when cytochrome P-448 was incubated with fetal liver microsomes, kidney microsomes, and cumene hydroperoxide pretreated hepatic microsomes from female and male rats, respectively. This study suggests that 21 day fetal AHH activity is low due to the low cytochrome P-450 (P-448 form) content. The lipid and NADPH-cytochrome c reductase present in fetal liver microsomes are capable of supporting much higher rates of AHH activity after incubation with 1.0 nmol of exogenous cytochrome P-448. Future studies will develop a coupled microsomal activating/embryo culture system which may have mechanistic uses in experimental teratogenesis as well as being a possible in vitro teratogenecity test system. Experiments concerning the mechanism of control of hepatic ALA synthetase and heme oxygenase will be useful in understanding the molecular mechanism of hepatic porphyria.

PUBLICATIONS

Kitchin, K. T. and Woods, J. S.: 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) induction of aryl hydrocarbon hydroxylase in female rat liver. Evidence for *de novo* synthesis of cytochrome P-448. *Mol. Pharmacol.* 14: 890-899, 1978.

Kitchin, K. T. and Woods, J. S.: 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) Effects on hepatic Microsomal Cytochrome P-448 Mediated Enzyme Activities. *Toxicol. Appl. Pharmacol.* 47: 537-546, 1979.

ENDOCRINE LABS OF MADISON - Madison, Wisconsin 53707
(NIEHS N01-ES-6-2111)

TITLE: Effects of Environmental Agents on Male and Female Reproductive Tract Function

CONTRACTOR'S PROJECT DIRECTOR: Karen MacKenzie, Ph.D.

PROJECT OFFICERS (NIEHS): G. W. Lucier, Ph.D., Acting Chief, Laboratory of Organ Function and Toxicology
J. A. McLachlan, Ph.D., Head, Transplacental Toxicology Group, LET

DATE CONTRACT INITIATED: March 20, 1976

CURRENT ANNUAL LEVEL: $215,000

PROJECT DESCRIPTION

OBJECTIVES: A series of experiments are described which will provide information on the effects of 36 selected xenobiotics (10 per annum) on the fertility of mice (male and female) following prenatal or postnatal exposure, and determine if levels of reproductive hormones have deviated from the norm or if a pathologic condition exists in mice whose reproductive capacity has been altered. Information will be obtained on the possible developmental toxicity of these chemicals, including transplacental carcinogenesis.

METHODS EMPLOYED: The approach that we are using to assess transplacental toxicity involves three phases of investigation. Phase I involves a preliminary screen to determine the "critical dose", which is defined as the highest dose that does not cause significant fetotoxicity or teratogenicity. Pregnant CD-1 mice are used as the test animal and chemicals are administered on days 10 through 16 of gestation. When generating the value for the "critical dose," we attempt to collect meaningful teratogenic and fetotoxic data so that maximum experimental information can be obtained from the protocol. In the teratogenicity studies, two-thirds of the fetuses (19 days of gestation) from each litter are fixed in 95% methyl alcohol and examined for skeletal alterations. One-third of the fetuses are preserved in Bouin's fixative, sectioned, and examined for internal anomalies. Autopsy data include determinations of corpora lutea, implants, resorptions, viable fetuses, fetal body weight, and sex ratio.

Dose schedules for animals used in Phase II are; controls, one-half of the critical dose, and one-tenth of the critical dose. If no "critical dose" is established, doses of one-half and one-tenth the LD_{50} are employed. In Phase II studies, 24 pregnant females are used in each group and are administered vehicle or vehicle plus chemical on days 10 through 16 of gestation. One group of animals receives a dose of DES (10 mg/kg on days 10-16), which significantly reduces fertility of both male and female offspring and this group is used as a positive control. Parameters evaluated in the initial Phase II studies include fertility, gestation, viability, survival, and lactation indices. Necropsy examinations of F_1 males are made at eight weeks, six months, 12 months, and a terminal necropsy is also performed. F_1 males are examined at seven weeks and again at the termination of the experiment. Tissues examined for histological alterations include liver, kidney, lung, small intestine, spleen, thymus, pituitary, adrenal, thyroid, parathyroid, gonads, uterus, epididymis, cervix, vas deferens, vagina, seminal vesicles and prostate. All tissues are checked for gross lesions. Fertility of at least 20 F_1 males are evaluated at eight weeks and again at eight months of age. Two virgin females (3-10 weeks old) are placed with each male every five days for 25 days (total of 10 females per male). Twelve days after removal, the females are weighed, laparotomized and the number of fetuses (alive, dead, or abnormal) and resorptions recorded. Fertility of F_1 females is determined by a lifetime forced-breeding study. Fifty F_1 females are selected randomly from each treatment group and each female is cohabited with one proven breeder male. The F_1 females remain with the males and are allowed to deliver at term. The number of F_2 young (alive and dead) are recorded and the young examined for gross anomalies. Litters are examined again on days 4 and 20. New breeder males are replaced at 180-day intervals. Plasma samples are taken prior to necropsy for analysis of progestins, androgens, and estrogens in groups of treated animals exhibiting deficits in reproductive tract function. The estrous cycle is monitored in eight week old F_1 females.

Although emphasis is placed on reproductive tract function of F_1 offspring, it would seem wasteful and narrow-minded to ignore manifestations of impaired organ function of other systems. Accordingly, the offspring are monitored for behavioral parameters, immunological competence, transplacental carcinogenesis, and cardiovascular function.

The objectives of Phase III are to determine dose-response relationships of chemicals exhibiting transplacental toxicity, to elucidate mechanisms of toxicity, to characterize structure-activity relationships, and to define the role of perinatal pharmacokinetics in transplacental toxicity. Of course, the protocols for Phase III studies cannot be developed until at least portions of Phase I and Phase II studies are completed. Many of the Phase III studies are conducted at the NIEHS.

MAJOR FINDINGS AND PROPOSED COURSE: Chemicals investigated or planned for study include DES, TCDD, DES + TCDD, Zearanol, Zearalenone, Mirex, Kepone, DDE, Methoxychlor, Hexabromobiphenyl (6-BB), Hexachlorobiphenyl (6-CB), Tetrachlorobiphenyl (4-CB), DMBA, 3-Methylcholanthrene, Benzpyrene, Dichloropropene, Phosphamidon, Dicrotophos, Atrazine, Aldicarb, and Carbaryl. Completed protocols for reproductive capacity following treatment with these chemicals are not available since these are lifetime studies. The fetotoxic and/or teratogenic dose has been established or will be established for each of these compounds in the CD-1 mouse. For example, Zearanol, a synthetic Mycotoxin analog, was shown to cause skeletal abnormalities at a dose of 300 mg/kg/day on days 10-16 of gestation. The estrogenic potency of this compound was determined to be approximately 1/600 that of DES. However, reproductive capacities of these offspring were unaffected. Localization of zearanol/zearalenone in specific fetal tissues will be performed by radioimmunoassay procedures.

Marked and unusual behavioral changes were observed in offspring from 4-CB treated (32 mg/kg/day) pregnant mice by two to three weeks after parturition. Increased locomotor activity and general restlessness were noted in approximately half of the surviving litters. General symptoms included characteristic jerking or rotational movement of their heads. There were also episodes of constant circling movement which seemed to be initiated by a loud noise. All offspring in an affected litter exhibited behavioral alterations and the symptoms were evident at least through eight months of age. Affected animals gained weight at approximately the same rate as controls and the survival rates of rotating and non-rotating offspring in the treated group were not different. Moreover there was no correlation between hydronephosis and behavioral alterations and the occurrence of hyperactivity was not sex-related. The complexity of the symptomology indicates damage to the central nervous system. Although conventional histological techniques did not find microscopic alteration, electron micrographs revealed CNS outgrowths into the peripheral nervous system. This lesion resembles those observed in the Werdnig-Hoffman syndrome. Foster mother experiments demonstrated that the effect is related to prenatal exposure to 4-CB.

Tissue distribution studies of ^{14}C-4-CB in CD-1 mice revealed some important findings. We observed that only very low levels of ^{14}C-4-CB were detected in weanlings following exposure to ^{14}C-4-CB via both placental transfer and suckling. Radioactivity was not detectable in eight week-old offspring indicating that behavioral alterations occur in the absence of the chemical. These studies also revealed that newborn tissues had, in general, higher 4-CB concentrations at parturition than did maternal tissues. This finding may help explain the occurrence of fetotoxicity and teratogenicity in 4-CB treated groups whereas no such effects were found in 6-CB and 6-BB treated animals.

It also appears that mice exposed to 4-CB prenatally have decreased reproductive capacity.

Studies with the polycyclic aromatic hydrocarbon (PAH) dimethylbenzanthracene (DMBA), an environmental carcinogen, have shown that gestational treatment with as little as 1.25 mg/kg results in absence of germ cells in both male and female offspring at seven weeks of age. Other abnormalities were not noted; these effects could not be induced with other PAHs, 3-methylcholanthrene or benzpyrene at doses 50 fold higher than that for DMBA.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Environmental chemicals can cause reproductive toxicity in mammals which is manifested in decreased fertility. However, these chemicals might affect male and female reproductive processes differently since oogenesis is an entirely prenatal event and spermatogenesis occurs throughout adult life in most eutherian mammals. The possibility exists that lesions of the reproductive tract which occur in utero might result in long term impairment of the reproductive tract in females and have a totally different effect on the male reproductive process.

The objective of the proposed study is to determine the effects of environmental chemicals on the reproductive capacity of male and female mice. Reproductive capacity is to be evaluated by the repetitive forced breeding method for females and by a serial mating technique for males. Since those animals are held for 18 months, developmental toxicities of other organ systems are noted including the CNS, immune and cardiovascular systems. The potential for transplacental carcinogenesis is also noted.

These studies are done in such a way as to support existing programs in the Laboratory of Environmental Toxicology concerned with developmental toxicity and transplacental toxicity. The purpose of the contract is two-fold; to identify environmental chemicals that have potential developmental toxicity emphasizing reproductive tract lesions; and to identify chemicals to Intramural scientists that are toxic and need to be studied from a mechanistic viewpoint.

PUBLICATIONS

Lucier, G. W., Davis, G. J. and McLachlan, J. A.: Transplacental toxicology of the polychlorinated and polybrominated biphenyls. In Mahlum, D. D., Sikov, M. R., Hackett, P. L. and Andrew, F. D. (Eds.): Proceedings of the Conference on the Developmental Toxicology of Energy Related Pollutants. Department of Energy Symposium Series 47: 1978.

Tilson, H. A., Davis, G. J., McLachlan, J. A. and Lucier, G. W.: The effects of polychlorinated biphenyls given prenatally on the neurobehavorial development of mice. Environ. Res. 18: 466-474, 1979.

Chou, S. M., Miike, T., Payne, W. M. and Davis, G. J.: Neuropathology of spinning syndrome induced by prenatal intoxication with a PCB in mice. Ann. N. Y. Acad. Sci. (In press).

NATIONAL MARINE FISHERIES SERVICE - Beaufort, North Carolina 28516
(Interagency Agreement Between NIEHS & NOAA)

TITLE: The Physiological Effects of Arsenic, Cadmium, and Copper on Marine Shellfish

CONTRACTOR'S PROJECT DIRECTOR: David W. Engel, Ph.D.

PROJECT OFFICER (NIEHS): B. A. Fowler, Ph.D., Research Biologist
Renal and Intracellular Function and Toxicology Group

DATE CONTRACT INITIATED: July 23, 1975

CURRENT ANNUAL LEVEL: $50,000

PROJECT DESCRIPTION

OBJECTIVES: This study was undertaken to determine the physiological, biochemical, and ultrastructural effects of arsenic, cadmium, and copper on tissues of marine shellfish in comparison with those previously observed in mammals. Evaluation of pharmacokinetic and toxicological differences in comparison to similar doses of Cd administered as mammalian cadmium-thionein, oyster cadmium-binding protein or Cd^{+2} is to be accomplished.

METHODS EMPLOYED: Flowing seawater systems with continuous trace element injection, atomic absorption spectroscopy, radiotracer analyses, histological and ultrastructural techniques, cellular respiration studies, and proton-induced X-ray emission analyses.

MAJOR FINDINGS AND PROPOSED COURSE: Physiological, Ultrastructural and Biochemical Findings. The two most significant findings are concerned with the isolation and characterization of an apparently inducible cadmium-specific metal binding protein from oysters exposed to cadmium in seawater. This protein which is chemically different from mammalian metallothionein seems responsible for the known bioaccumulation of cadmium by these organisms and appears to play a role in determining cadmium toxicity to these animals. A second area where progress has been made concerns the role of concretions and metal-binding proteins in kidneys of edible shellfish. The renal concretions have proven useful both in terms of explaining intensive accumulation of ^{54}Mn from fallout in bivalves but also in providing good biological standards for X-ray microanalysis since in situ metal analyses may be checked against analytical values derived by atomic absorption spectroscopy on concretions isolated by centrifugation from the same animals. Further ultrastructural/biochemical studies on the mechanisms of copper and cadmium toxicity to shellfish are in progress.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies will provide a basis for evaluating mechanisms of uptake and cellular responses to chronic low level trace metal exposure in tissues of relatively simple organisms in comparison to those observed in mammals.

Data generated from this effort should also prove useful in evaluating differences in trace metal pharmacokinetics and toxicity following ingestion of metal contaminated marine shellfish. The future course of the project will involve the specified objectives of this interagency agreement in relation to understanding the subcellular mechanisms of trace element uptake and toxicity in marine organisms in comparison to mammals. This arrangement also provides a mechanism for estimating potential human risk following ingestion of metal contaminated marine shellfish.

PUBLICATIONS

Engel, D. W. and Fowler, B. A.: Factors influencing the accumulation and toxicity of cadmium to marine organisms. Environ. Hlth. Perspec. 28: 81-88, February 1979.

Engel, D. W. and Fowler, B. A.: Copper and Cadmium-Induced Changes in the Metabolism and Structure of Molluscan Gill Tissue. In: Marine Pollution; Functional Response. W. B. Vernberg, A. Calabrese and F. Thurberg (eds.) Academic Press, New York (in press).

FISHERIES RESEARCH INSTITUTE - Univ. of Washington, Seattle, Washington
(NIH-NO1-ES-7-2101)

TITLE: Carcinogenic Effects of Petroleum Hydrocarbons on Selected Marine and Estuarine Organisms

CONTRACTOR'S PROJECT DIRECTOR: Sam P. Felton and Bruce S. Miller

PROJECT OFFICERS (NIEHS): J. R. Bend, Ph.D., Visiting Scientist
Laboratory of Pharmacology
B. A. Fowler, Ph.D., Research Biologist
Laboratory of Organ Function and Toxicology

DATE CONTRACT INITIATED: December 16, 1976

CURRENT ANNUAL LEVEL: $92,556

PROJECT DESCRIPTION

OBJECTIVES: To compare incidence of tumors, particularly epidermal papillomas, in exposure and control groups of selected flatfish species in response to exposure to benzo(a)pyrene (BaP) and/or its electrophilic derivatives and to qualitatively examine the histopathology of comparable tumors occurring in both exposure and control groups.

METHODS EMPLOYED: Static and flowing sea water systems with aquaria and sand coated with BaP. Monitoring of BaP concentrations by gas chromatography, assay of aryl hydrocarbon hydroxylase (AHH), examination of fish tissue by routine histopathological procedures.

MAJOR FINDINGS AND PROPOSED COURSE: Progress to date has shown that exposure of young flatfish to BaP in water (1 μg/l or by intraperitoneal injection of 2.5, 5 or 10 mg/kg) produces an initial induction of liver AHH activity which declines by 4 and 6 months to levels below those of controls. Studies now in progress are concerned with elucidating the nature of the wide inter-animal response to BaP and possible relationships to carcinogenesis in the animals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies should provide fundamental information about the metabolism and biological responses of marine organisms consumed as food to a carcinogenic petroleum hydrocarbon. Results of the study should specifically yield useful data on the relationship between AHH activity and the carcinogenic susceptibility of marine organisms. The proposed course is to expose flatfish to BaP and examine the relationship between AHH activity and carcinogenesis in these species.

PUBLICATIONS

Felton, S. P., Iwaoka, W. T., Landolt, M. L. and Miller, B. S.: Techniques for water-borne administration of benzo(a)pyrene to aquatic test organisms. Proc. Symp. "Carcinogenic Polynuclear Aromatic Hydrocarbons in the Marine Environment." U.S.E.P.A. (In press)

Landolt, M. L., Felton, S. P., Iwaoka, W. T., Miller, B. S., DiJulio, D. and Lloyd, B.: Bioaccumulation and toxicity in English sole (*Parophrys vetulus*) following water-borne exposure to benzo(α)pyrene. Proc. Symp. "Carcinogenic Polynuclear Aromatic Hydrocarbons in the Marine Environment." U.S.E.P.A. (In press)

LABORATORY OF MOLECULAR GENETICS

THE LABORATORY OF MOLECULAR GENETICS
Summary Statement

The Laboratory of Molecular Genetics (LMG) was formed in FY-79 by the splitting of the Laboratory of Environmental Mutagenesis into the LMG and the Laboratory of Animal Genetics. It is comprised of two research groups, Molecular Genetics and the Environmental Mutagen Test Development Program (EMTDP). The dual aim of the LMG is the elucidation of mutational mechanisms at the molecular level in both prokaryotes and eukaryotes using both genetic and biochemical techniques; and the development and application of practical mass-screening programs to detect mutagens among diverse foods and environmental chemicals. The growing recognition over the past decade of the potential role of environmental chemicals in the etiology of much modern disease has stimulated considerable public and professional interest in the general problem of environmental mutagenesis. There has been remarkable public awareness of and concern for the importance of protecting the human genome from genetic damage that would constitute a legacy of ill health for many generations into the future.

MOLECULAR GENETICS

This newly formed group focuses primarily upon the diversity of molecular mechanisms which underlie the mutation process, starting with the induction of lesions in DNA and proceeding through various mutation avoidance and mutation fixation processes.

Bacteriophage T4 is a highly adaptable and useful model system for the study of mutagenesis, and is being used to study the process of "heat" mutagenesis. Heat is an ever-present mutagen and a potentially important contributor to "spontaneous" mutation in mammals; furthermore, the chemical reactions which heat triggers in DNA are also likely to be enhanced by certain chemicals. One mechanism of heat mutagenesis involves cytosine deamination, producing uracil which in turn behaves like thymine. Ordinary phage T4 DNA contains 5-hydroxymethylcytosine instead of ordinary cytosine, and a part of the group's efforts now centers on the question of the relative importance of the two forms of cytosine in this mutagenic mechanism. Another mechanism of heat mutagenesis involves the conversion of guanine to a pyrimidine analogue, for which a model exists: glycosidic bond migration from the N^9 to the N^2 position of guanine, producing "neoguanosine". Conditions are now being explored for the synthesis of deoxyneoguanosine triphosphate and its incorporation into defined DNA polymers where its coding properties can be studied.

At least two classes of mutation avoidance appear to operate to protect genomes against heat mutagenesis (which introduces an estimated 100 or more premutational lesions into each human cell every day). One, excision of the damaged base, is being sought in the particular case of neoguanosine, and is already known to exist in the case of uracil. The other mechanism is poorly understood, but appears to circumvent the miscoding properties of the lesion without removing it from DNA by conventional means.

The role of DNA polymerases in maintaining the fidelity of DNA synthesis is being studied in two model systems. In the first, the highly mutagenic base analogue 2-aminopurine is being examined to distinguish two kinds of errors in which it participates: errors of insertion in place of adenine, and subsequent mispairing errors involving pairing with cytosine. Using a variety of mutant DNA polymerases, it has proven possible to determine the relative role of these two processes to the overall mutation frequency. The second mode of analysis involves the study of base analogue incorporation *in vitro*, in which the ability of the polymerase to distinguish between adenine and N6-methyladenine is being probed.

Different organisms, and even different genes within an organism, display greatly different mutation rates, both spontaneously and in response to exogenous chemicals. Among the determinants of this variation is the DNA base pair sequence itself: neighboring base pairs markedly affect mutation rates of a given base pair. A system has been discovered in which such a modulation of mutation rate is observed due to a sequence configuration which is decidedly distant from the affected sequence, a distance estimated from genetical measurements to be about 30 base pairs (too far for typical electronic interactions). The underlying nature of this effect is currently being analyzed by means of DNA base pair sequence analyses of the region.

A major fraction of chemical mutagenesis is mediated in most organisms by error-prone or mutagenic repair. The several genes responsible for this process in phage T4 are being identified and characterized in detail. Means have recently been developed to separate these genes into different, sequentially acting steps, thus providing a good basis for their biochemical analysis in the near future.

A major determinant of DNA topology, and thereby of DNA mutability, is DNA gyrase. This complex enzyme system has been studied in great detail, making use of the fact that resistance to a number of antibiotics is mediated by mutations which alter the components of DNA gyrase. These subunits appear to carry out different and multiple roles, and have now been identified to varying degrees in the bacteria *E. coli* and *B. subtilis* and in phage T4.

DNA replication generally begins at a unique region, the origin. As an initial step in the analysis of DNA replication in higher eukaryotic cells, but one making use of a suitable model system, the origin of DNA replication has been studied in the mitochondria of *Drosophila*. Its location has been determined by restriction mapping, and sequencing and further characterization should now be possible.

MUTAGEN SCREENING

The EMTDP is a congressionally mandated program which ultimately aims to survey very large numbers of chemicals for mutagenic activity in a modified tier approach, the first level of which is comprised of bacterial tests and, to the extent possible with available national resources, *in vitro* cytogenetic assays. Cultured mammalian cell specific-locus mutational assays may also be added in the next few years. A selection of chemicals, including those positive in the lower levels, will also be subjected to *in vivo* animal testing, beginning presently with Drosophila. After many years of preparatory work, the first three microbial screening contracts have been awarded. This fiscal year will also see Drosophila recessive lethal and heritable translocation screening contracts awarded, and perhaps some preliminary contracts to explore the suitability of various mammalian cell mutation systems.

Two major support contracts for these activities are also now well under way. A contract has been awarded for the establishment of a chemical repository, at which designated chemicals are procured, characterized to some extent, and distributed to the testing laboratories as coded samples with minimal necessary information. A computer-based data management system is under development, and should be available by about the time that large amounts of data begin to accumulate in the program.

The EMTDP staff maintain vigorous research programs in parallel with their contract management activities. Numerous aspects of microbial mutagen response and mutagen activation are continually explored; this year, for instance, the mutagen sodium azide, a common positive control substance in this type of testing, was discovered to release a gaseous, readily diffusable mutagenic derivative capable of reaching other plates in an incubator.

Mutational modifications of microbes have long been known which alter the microbe's response to chemical mutagens, sometimes increasing and sometimes decreasing the mutagenic response. Mutants of Drosophila have very recently become available which exhibit increased sensitivity to the killing effects of certain chemical mutagens, and the mutational responses of these strains are now being surveyed. Some such strains may display increased sensitivity to mutagens, and might thereby lead to increased sensitivity in mutagen screening programs.

The most recent addition to NIEHS mutagen screening activities is a contract with the Lawrence Livermore Laboratory to examine the question of mutagenic activities introduced into foods by processing procedures, especially cooking. It has been evident that activities defined by Ames-system responses are introduced by certain modes of cooking of foods, particularly protein-rich foods, and that a number of different chemical mechanisms and mutagenic agents are involved. A systematic survey based on actual food preparation and consumption practices is badly needed, together with a careful assessment of mutagenic activities in a variety of assay systems, including the intact mammal. To date, this contract has confirmed some of the previous claims in this area, and has developed superior methods for the extraction of active components from meat cooked at moderate temperatures (200°C).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60052-03 LMG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Environmental Mutagenesis Test Development Program

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Zeiger	Research Microbiologist	LMG	NIEHS
Other:	D. Walters	Chemist	EBCB	NIEHS
	B. Margolin	Statistician	BB	NIEHS
	J. Mason	Staff Fellow	LAG	NIEHS

COOPERATING UNITS (if any)
Laboratory of Animal Genetics, NIEHS
Environmental Biology & Chemistry Branch, NIEHS
Biometry Branch, NIEHS

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of the Environmental Mutagenesis Test Development Program are twofold. The first is the development of an integrated program for the routine testing of large numbers of chemicals for mutagenicity. This includes such diverse factors as test-system validation, identification and development of new test methods, development of an increased understanding of mutagenesis and development of a strategy and an organizational structure for conducting a large scale testing program and interpreting the results obtained. The second objective is implementation: to test up to 1000 commercial and environmental chemicals per year for mutagenicity.

PHS-6040

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: Mutagenicity testing: Testing has just been initiated in 3 contract laboratories using the Salmonella test system of Ames. These laboratories are: SRI International, Menlo Park, California; EG&G Mason Research, Rockville, Maryland; and Case Western Reserve University, Cleveland, Ohio. Each laboratory will test 75 coded chemicals during the first 12 months of the contract.

Chemicals: A list of 25 control chemicals and 225 chemicals for testing has been developed by an NIEHS Chemical Selection Committee. A contract was also awarded to Radian Corporation, Austin, Texas to develop a chemical repository and analytical facility. This contractor will store, dispense, encode, and ship the test chemicals to the testing laboratories. The repository will maintain an inventory which will include such information as amount of chemical in stock, lot number, synomyms, CAS number, Wiswesser Linear Notation, physical properties, analytical information, toxicological information and information on handling and decontamination procedures. This laboratory will also perform chemical analyses for identity and purity on all mutagenic substances.

Data Handling: Work is progressing as part of the NIEHS intramural program to develop statistical methods for analysis of microbial mutagenicity data. Efforts are underway to develop a computerized data base management system which will be able to collect, store and evaluate experimental information and data. This system will enable project officers to reconstruct the experiments, retrieve data, monitor the levels of effort and quality of work, summarize data across laboratories, and perform statistical operations on the data.

Until this system is working, microbial testing contractors will use a microbial mutagenesis data storage and retrieval system developed for the NCI In Vitro Program and administered under contract by EG&G Mason Research, Inc., Rockville, Maryland.

Future Contracts: Two or three contracts will be awarded this year to test unknowns for their ability to induce chromosome aberrations and sister chromatid exchanges in Chinese Hamster Ovary cells in culture.

Two additional contracts will be awarded for the testing of substances which are mutagenic in vitro in Salmonella in Drosophila for the induction of recessive lethal mutations and reciprocal translocations.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There are thousands of chemicals in use and the number is ever increasing. The majority of these substances has not been evaluated for the potential to induced heritable genetic alterations; and, hence, the possible risk to future human generations is unknown. This program will develop an efficient coordinated system for mutagenesis testing and mutagenesis data management and should serve as a model system. The data obtained from this program will be useful for decision making on the safety of various classes of chemicals to which man is exposed.

PUBLICATIONS

Zeiger, E. & J. W. Drake, An Environmental Mutagenesis Test Development Program, _in_ IARC Publications _in press_.

Zeiger, E., Mammalian cell mutagenesis in a mutagen screening program, _in_ Banbury Report No. 2; Quantitative Mammalian Cell Mutagenesis and Mutagen Screening _in press_.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60077-02 LMG

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Molecular Mechanisms of Heat-Induced Mutation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. S. Ripley	Senior Staff Fellow	LMG	NIEHS
Other:	Danna Sherrick	Summer Graduate Student	LMG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Heat-induced mutations in bacteriophage T4 have been demonstrated to occur by at least two distinct mechanisms: (1) G:C base-pairs are converted to A:T base-pairs (transversion). The C of the base-pair is the target of the first mechanism. The G of the base-pair is the target of the second mechanism. Studies of the step(s) required for conversion of the heat-induced lesion (pre-mutational lesion) to the fully expressed mutation are continuing. The specificity of the transversion reaction (G:C to T:A versus G:C to C:G) is to be determined. T4 phage contain glucosylated hydroxymethylcytosine in their DNA. The influence of this modification on mechanism (1) will be explored.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard genetic manipulations in bacteriophage T4 are used. Mutations are measured in the rII genes. Appropriate mutants are available for making T4 with DNA which is non-glucosylated or which has C rather than hydroxymethyl-C. To distinguish G:C-to-T:A from G:C-to-C:G transversions, a missense mutant having a serine (UCA) or a tyrosine (UAC) codon is needed.

MAJOR FINDINGS AND PROPOSED COURSE: In vivo measurement of mutation when C is substituted for glucosylated hydroxymethyl-C (glu-HMC) in heat-induced G:C to A:T transitions shows a lower frequency of detected mutations. The rate of mutation as a function of temperature or time at constant pH is decreased several-fold. The lower frequency of mutations may reflect one or both of two separate aspects of the DNA. One aspect is chemico-structural differences between C and glu-HMC DNA's. An example of a mechanism which might lower the frequency of mutation is a differential rate of deamination in C versus glu-HMC DNA at constant pH. In vitro measurements of the rates of deamination are proposed in conjunction with pH profiles of the reaction both in vivo and in vitro. The second aspect which might lower the frequency of mutation in C DNA involves possible differences in the metabolic processing of C and glu-HMC in the DNA or their mutagenic intermediates. If deaminated C (i.e. U) is indeed the mutagenic intermediate, accurate repair acting on U but not glu-HMU could give a reduced mutation frequency.

The host, E. coli, is known to possess an enzyme activity which removes uracil from DNA. The enzyme, uracil-N-glycosylase, depyrimidates uracil in DNA but does not depyrimidate HMU in DNA. E. coli mutants deficient in this enzyme are called ung. C-containing T4 DNA shows the same mutation frequency on ung^- and ung^+ hosts, suggesting that this enzyme is not responsible for the lowered mutation frequency in C DNA; but these experiments are not definitive since residual enzyme activity may be present in the ung^- mutant (less than 1% has been reported).

C-containing DNA is an unnatural form for T4 DNA and many differences between C and glu-HMC DNA may exist relating to the structure of the DNA in the phage head during heat treatment and to differential metabolism during growth of the phage.

The role that glucose alone may play in mutagenic differences between DNA's is just coming under investigation. The appropriate triple mutants are being constructed. These will then be heated to measure their reversion responses in the presence or absence of glucose.

The nature of mispairing of the heat-induced transition intermediate in vivo can now begin following upon the acquisition of adequate facilities (a warm room) and technical support (summer graduate student).

Previous genetic attempts to confidently discern the pathway of heat-induced transversion mutations (G:C to T:A versus G:C to C:G) have met with failure. I propose to sequence an amber mutant and rUV74, a missense mutant, and their heat-induced revertants, to determine the major product of heat-induced transversion mutation. Appropriate rII mutants having C DNA have been constructed. Heat mutagenesis will be done to collect revertants in the C-DNA background. The C-DNA can be restricted and cloned on a lambda plasmid. Sequencing of these plasmids in the region of interest will be carried out collaboratively with sequencing already planned in a nearby region of the rII genome.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Definition of the pathway of reversion is important because an important model for the mutagenic intermediate (neo-G) would be seriously challenged by the result that G:C to T:A as well as G:C to C:G was induced by heat.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60078-02 LMG

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

T4 DNA Polymerase (Gene 43) Error Rate with DNA Containing 2-Aminopurine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. S. Ripley	Senior Staff Fellow	LMG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This is a study of the error rates of mutator, anti-mutator and wild type T4 DNA polymerase (gene 43) in 2-aminopurine-substituted DNA in vivo. Base analogue-induced mutation rates can be influenced both by the degree of incorporation of the analogue into the DNA and by the frequency of errors of replication past incorporated 2-aminopurine. An appropriate method is being sought to separate the incorporation from the replication step of mutagenesis by 2-aminopurine. Preliminary evidence suggests that separation of incorporation and replication errors may be achieved by mixed infection with one phage, substituted with 2-aminopurine and carrying one allele of gene 43, and a second, unsubstituted phage which supplies a second allele (mutator, anti-mutator or wild type) of gene 43. Quantitative analysis of mutagenesis in these mixed infections may allow determination of error rates in vivo for different gene 43 alleles independent of incorporation rates.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard genetic manipulations in bacteriophage T4 utilizing the well characterized mutants of the rII genes to measure mutation rates and well characterized alleles of T4 DNA polymerase which have been shown *in vivo* and *in vitro* to have altered fidelity of DNA replication.

MAJOR FINDINGS AND PROPOSED COURSE: The preliminary evidence has been confirmed and relative rates of mutation resulting from replication by anti-mutator, wild type and mutator alleles past template 2AP have been measured. The measurement depends upon a constant fraction of polymerases acting *in trans* on a normal template compared to a template containing 2-aminopurine. Control experiments confirmed constant *trans* activity.

An unusual phenomenon was observed with respect to the amount of *trans* activity in the particular case of tsL56 mutator against wild type polymerase but not against tsCB87 antimutator. In this case the mutator seems to act *trans* in about 90% of the replications compared to 40-50% in all other combinations tested. Further investigation will attempt to relate this phenomenon to gene 43 levels or to the processiveness of the mutator protein.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An understanding of the molecular mechanisms by which the DNA replication machinery regulates the level of mutation in an organism is necessary for a basic understanding of mutation, inherrant error rates in DNA replication, and errors of replication due to the impact of the environment upon the organism. This study adds to our knowledge of how a DNA polymerase distinguishes aberrant DNA constituents.

PUBLICATIONS

Ripley, L. S.: *In vivo* replicate of templates containing 2-aminopurine by T4 polymerases. In preparation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60080-02 LMG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Modification of Site-Specific Mutation Rates by a Nearby Base-Pair Substitution

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. A. Conkling	Geneticist	LMG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Some evidence supports the hypothesis that the mutation rate at a particular site is influenced by neighboring base-pairs. I have sought to modify the reversion and conversion frequencies of bacteriophage T4 rII nonsense codons by inserting nearby temperature-sensitive rII lesions. The insertion of a ts mutation reduces the 2-aminopurine-induced reversion of an rII amber mutation about three-fold. The ts mutation reduces the corresponding UAA→UGA conversion about eight-fold, while the reversion of the ochre codon to glutamine (UAA→CAA) is not affected. Selection controls show no measurable selection against the ts marker.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Double rII mutants have been constructed composed of one of two homologous nonsense mutants (rHB129am and rHB129oc) plus a ts mutation (rPS78). The spontaneous and 2-aminopurine-induced reversion and conversion frequencies of the nonsense mutations are measured in the presence or absence of the ts mutation.

MAJOR FINDINGS AND PROPOSED COURSE: The spontaneous and 2-aminopurine-induced reversion frequencies of rHB129am are reduced about threefold in the presence of rPS78. The modified reversion frequency does not appear to reflect an altered efficiency of detection of revertants, but rather an alteration in the mutation rate itself. The 2-aminopurine-induced reversion of rHB129oc is not affected by the ts mutation. The 2-aminopurine-induced conversion of rHB129oc to rHB129am is reduced approximately three-fold in the presence of the ts mutation. The 2-aminopurine-induced conversion of rHB129oc to rHB129op is reduced about eight-fold in the presence of the ts mutation. Genetic evidence suggest that about 30 base-pairs separate the rHB129 site from the rPS78 site. The precise physical distance will be determined by molecular cloning and DNA sequencing of this region of the T4 rIIA gene.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The characteristic mutation rate of a genetic site is often supposed to depend not only upon the base-pair at that site, but also upon the molecular environment determined by nearby base-pairs. This idea seems to have originated as an explanation of mutation "hot spots". A rigorous demonstration of a neighboring base effect requires that a mutation rate be altered as a result of a nearby base-pair substitution. An understanding of the fundamental mechanisms of mutagenesis requires an understanding of the role of the molecular environment upon mutation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60081-02 LMG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Genetics of the Misrepair Mutagenesis System of Bacteriophage T4

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. A. Conkling	Geneticist	LMG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Lesions induced in DNA may be repaired by "error-proof" or "error-prone" repair mechanisms. Error-prone systems repair the lethal damage of the lesion, but in doing so may fail to restore the original nucleotide sequence, producing mutations. This error-prone process is called misrepair mutagenesis. Bacteriophage T4 has its own misrepair pathway, mediated by at least three genes, x, y and w. Mutations in two of these genes, x and y, suppress mutations of gene 49, a gene involved in DNA packaging. This allows the selection of alleles of x and y. ts alleles of x and y have been selected. These are temperature sensitive both for suppression of gene 49 amber mutants and for UV sensitivity. Amber alleles of x and y are being sought. Using these and other x and y alleles, the genes x and y will be mapped. The ts alleles will be used to study the time course of mutagenesis in T4. Finally the enzymology of the T4 misrepair system will be studied by genetic and biochemical approaches.

PROJECT DESCRIPTION

METHODS EMPLOYED: Suppressors of gene *49* amber mutations will be selected. These can be arranged into complementation groups using a complementation test based on UV sensitivity. Their sensitivities to inactivation and mutagenesis by UV irradiation will be determined.

MAJOR FINDINGS AND PROPOSED COURSE: Mutations in genes involved in T4 misrepair mutagenesis result in decreased recombination frequencies, increased sensitivity to inactivation by UV or gamma irradiation, and decreased sensitivity to mutagenesis by UV or gamma irradiation. Mutations in genes *x* and *y* can suppress amber and *ts* mutations of gene *49*, a gene involved in DNA packaging. *ts* alleles of *x* and *y* have been isolated. These are *ts* both for suppression of gene *49* amber mutations and for sensitivity to inactivation by UV irradiation. Preliminary evidence suggest that the suppression of the gene *49* defect is an early function. This might provide an assay for the *x* and *y* gene products *in vitro*. Amber alleles of *x* and *y* are being sought. With these, the *x* and *y* gene products might be visualized on two-dimensional gels. Temperature shift experiments using the *ts* alleles will allow a study of the time-course of mutagenesis. The genes *x* and *y* will be mapped using alleles selected by their ability to suppress gene *49* mutants.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Although the misrepair pathway is thought to be a major pathway of mutagenesis in eucaryotic systems, the biochemistry of this repair process is not understood. Bacteriophage T4 offers a simple model system to begin to understand the enzymology of the misrepair process and also offers a highly defined genetic system to study mutagenesis along certain defined pathways.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60082-02 LMG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Neoguanosine as a Naturally Occurring Mutagenic Intermediate

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. C. Mace	Staff Fellow	LMG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project will investigate the possibility that deoxyneoguanosine residues in DNA (hypothesized to be the G:C→C:G transversion pathway intermediate for heat mutagenesis in T4 bacteriophage can indeed form a base pair with a deoxyguanosine residue on the opposing strand of a double helix. The formation of this novel base in polymeric and monomeric form is being studied. Its capacity to actually form a base pair with guanosine residues will be approached by both physical and biochemical methods.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Column chromatographic and thin layer chromatographic schemes have been worked out for efficient separation of neoguanylic acid from guanylic acid as well as from a variety of other heat products formed under certain conditions. These methods have been employed to isolate substantial amounts of the desired product as well as to study the rate and mechanism of its formation under a variety of experimental conditions. Established techniques are being used to convert the monophosphate to the di- and triphosphate. A direct chemical synthesis of neoguanosine will also be attempted and if successful will allow more convenient preparation of large amounts of the compound. Detection of neoguanosine in DNA at this time depends on radioactive labeling of the initial material, enzymatic degradation and thin layer separation of the resultant products. Alternate approaches to be attempted include preparation of specific antibody to neoguanosine from rabbits injected with neoguanosine chemically coupled to protein carrier molecules. Finally, direct chemical synthesis will allow synthesis of heavy-isotope-labeled neoguanosine and open the possibility of mass spectral analysis of deoxyguanylic acid in natural DNA's.

MAJOR FINDINGS AND PROPOSED COURSE: Neoguanylic acid has been isolated in large amounts and is being chemically and physically characterized. The basic conditions for formation have been examined; these show its formation to be closely associated with acid catalyzed depurination. Experiments designed to distinguish between an intermolecular and intramolecular reaction are also being conducted. In the immediate future, a large batch of deoxyneoguanylic acid will be prepared for its detailed analysis and use as starting material for the synthesis of labeled and unlabeled deoxynucleotide triphosphates. These can then be used for in vitro polymerization reactions and syntheses of polymers containing deoxyneoguanosine residues. Finally, detection of neoguanylic acid in DNA and RNA, enzymatic degradation and thin layer chromatographic analysis is being pursued. Protein-conjugated neoguanylic acid (later conjugated deoxynoguanylic acid) is being prepared for rabbit injection in hope of obtaining neoguanylic-specific antibodies. If deoxyguanylic acid residues are formed in DNA, a search for repair systems will be undertaken.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The heat-induced G:C→C:G pathway could be a major contributor to the mutational load sustained by the mammalian genome. Thus, it seems important to determine if the hypothesized transversion pathway (involving a thermally induced migration of the glycosylic bond of deoxyguanosine) actually does occur and is able to form a base-pair with guanosine during the synthesis of DNA by T4 DNA polymerase. If the results indicate that deoxyneoguanosine is involved, investigations into the existence of this pathway and its repair will be pursued in mammalian cell systems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60084-02 LMG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Fidelity of Templated Polymerizing Enzymes During Polymerization

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. C. Mace	Staff Fellow	LMG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is to understand how templated nucleotide polymerizing enzymes achieve fidelity. Previous workers have concentrated on the 3'-to-5' exonuclease associated with many DNA polymerases. However, the mechanisms by which the polymerizing step achieves accuracy have been largely ignored. To investigate the latter, the effect of polymerase substrate analogues on the fidelity of the polymerization reaction are being studied. Modified nucleotide triphosphates will be utilized as probes of fidelity mechanisms. Initial efforts will focus on rigorously testing the assumed importance of classical Watson-Crick base pairing. Secondarily, additional kinetic steps will be probed with substrate analogues modified in the triphosphate moiety.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The preparation of modified deoxynucleoside triphosphates will be accomplished by techniques already established for ribonucleoside triphosphates. Standard methods will also be employed to characterize their utilization by the T4 DNA polymerase.

MAJOR FINDINGS AND PROPOSED COURSE: It has been reported that *E. coli* DNA polymerase I, during the course of *in vitro* DNA polymerization, used N^6-methyl deoxyadenosine triphosphate and deoxyadenosine triphosphate equally well. This result is surprising in view of NMR data showing that the *cis* rotational isomer of N^6-methyldeoxyadenine (i.e., the methyl group in the plane of the base and blocking Watson-Crick A:T base pairing) was favored by a twenty-to-one ratio over the *trans* rotational isomer (i.e., the methyl group in the plane of the base but oriented toward the N^7 position and therefore allowing Watson-Crick A:T base pairing). If base-pairing were important for preliminary selection of the triphosphate during polymerization the N^6-methylated substrate should have been used twenty-fold less efficiently than the unmethylated substrate. Clearly such a finding has major implications for our conception of the mechanism of DNA polymerase fidelity and must be further investigated with other DNA polymerases.

Therefore studies of T4 DNA polymerase fidelity mechanisms have begun with these two triphosphates. Thus far, it has been found that the T4 DNA polymerase does discriminate against the former of these two compounds in accordance with the simple expection from the relative proportions of the rotational isomers. Studies are continuing with this compound and shall be broadened to include other polymerases (e.g., *E. coli* pol I). Hopefully, the apparent contradiction between my result and that of Engel and von Hippel (vide supra) can be understood. Further probing the role of simple base-pairing for T4 DNA polymerase will be achieved by kinetically characterizing polymerization substrates which compete for incorporation opposite the same template nucleotide, but which are known to differ significantly in their hydrogen bonding strength to that template nucleotide (e.g., deoxyinosine triphosphate compared to deoxyguanosine triphosphate).

Later research will be aimed at investigating other fidelity mechanisms of T4 DNA polymerase. Again this will be approached with modified nucleotide triphosphates. My initial hypothesis is that a difference in catalytic rate for polymerization of a correct and incorrect triphosphate substrate can be the basis of discrimination at the polymerization step. Analogues which reduce the maximum catalytic efficiency would probably reduce the net polymerization rate difference attainable between a correct and incorrect (in respect to base pairing with the template) substrate. The net result would be a loss of enzyme fidelity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Understanding the mechanisms used by DNA polymerases to accurately replicate the template during the polymerization step of DNA synthesis has largely been ignored until now. Such experiments are fundamental to an understanding of how the *in vivo* replication of DNA can be accomplished with sufficiently few errors to maintain a biologically acceptable spontaneous mutation rate. Using modified and unmodified triphosphates and compairing the relative fidelities of incorporation, it is hoped that insights can be obtained. Finally, since an understanding of the mutation process is one part of the overall goal of this Institute, learning how DNA polymerases achieve their remarkable accuracy will certainly be an important step in that direction.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60101-01 LMG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Selection of a Computerized Data Base Management System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Zeiger	Research Microbiologist	LMG	NIEHS
	B. Margolin	Statistician	BB	NIEHS
Other:	T. Clemmer	Computer Specialist	BB	NIEHS

COOPERATING UNITS (if any)

Biometry Branch, NIEHS
National Institute of Neurological and Communicative Disorders

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The Environmental Mutagenesis Test Development Program (EMTDP) will be generating large volumes of data and experimental information on all chemicals which will be tested for mutagenicity. A computerized data base management system will be required to capture this information in an interactive mode in the testing laboratory, store, process, and analyze the data, and provide summary analyses to the experimenter and to the EMTDP project officers. This system should also allow the EMTDP staff to follow the course of testing with time in the large number of laboratories regardless of the mutagenicity test system being used. Efforts are underway in the selection and design of an acceptable system.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: An ad hoc advisory group consisting of Dr. Selma Kunitz (NINCDS, NIH), Dr. Frank Starmer (Department of Computer Science, Duke University) and Dr. Raymond Neff (School of Public Health, Harvard University) has been formed to aid the EMTDP and Biometry Branch staffs in definition of our computer needs and the identification or design of an acceptable system. An additional consultant was retained to survey existing non-commercial data base management systems to determine whether any met EMTDP's needs and to make recommendations to the advisory group.

MAJOR FINDINGS AND PROPOSED COURSE: The non-commercial data base management system survey identified one system (PROPHET) currently funded by DRR, NIH which may fit EMTDP's needs. The developers and purveyors of this system (Bolt, Beranek, and Newman, Inc., Cambridge, Massachusetts) have demonstrated the system and a decision will be made shortly on the adequacy of the system for EMTDP's needs.

If it is acceptable, work will be initiated to fit the system to the current contract information format. If it is not acceptable, a modification of the system may be attempted or EMTDP will initiate a contract for the development of a system that is adequate for the needs of the program.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This system will allow the rapid and accurate capture of testing data for generation of reports, data analysis, and for general contract management. It will greatly increase the efficiency of the testing program and will provide a vehicle for the efficient storage of large volumes of information.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60102-01 LMG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Testing of Chemicals of Interest in Salmonella

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Zeiger	Research Microbiologist	LMG	NIEHS
Other:	J. Guthrie	Microbiologist	LMG	NIEHS

COOPERATING UNITS (if any)
Laboratory of Animal Genetics, NIEHS
Environmental Biology & Chemistry Branch, NIEHS
Biometry Branch, NIEHS

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.05	0.20

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Salmonella typhimurium strains TA-98, TA-100, TA-1535, and TA-1537 are being used to test selected chemicals of interest for mutagenicity. Natalan, an antineoplastic drug which is mutagenic in eukaryotic cells was not mutagenic in these strains even at levels which produced cell killing. A series of cyclic hydrazides which are analogues of cyclic nitrosamines are being tested for mutagenicity for comparison with their mutagenic and non-mutagenic nitrosamine counterparts. N-aminopiperidine and N-aminomorpholine were both mutagenic, producing only base-pair substitution mutations in the presence and absence of rat liver S-9; however, more activity was seen in the absence of S-9. N-aminomorpholine was significantly more mutagenic than N-aminopiperidine.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The standard *Salmonella* plate test procedure of Ames was used with some modifications.

MAJOR FINDINGS AND PROPOSED COURSE: The hydrazide analogues of N-nitrosomorpholine and N-nitrosopiperidine (both potent rat carcinogens) are mutagenic. However, unlike the nitrosamines which have similar mutagenic activities, N-aminopiperidine is an extremely weak mutagen when compared to N-aminomorpholine.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Cyclic hydrazides are used as organic intermediates in organic synthesis reactions. This demonstration of their mutagenicity has implications for the health of workers handling these chemicals. The lack of mutagenicity of Natulan for the standard *Salmonella* tester strains, although it is positive in yeast and mammalian cells, demonstrates that a negative *Salmonella* test might not be sufficient to declare a substance non-mutagenic.

PUBLICATIONS

Bronzetti, G., E. Zeiger, and H. V. Malling. Genetic toxicity of procarbazine in bacteria and yeast. Mutation Res. in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60103-01 LMG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Development of New and Improved Salmonella Test Methods

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Zeiger	Research Microbiologist	LMG	NIEHS
Other:	I. Robertson	Visiting Fellow	LMG	NIEHS

COOPERATING UNITS (if any)
Laboratory of Animal Genetics, NIEHS
Environmental Biology & Chemistry Branch, NIEHS
Biometry Branch, NIEHS

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.1	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Salmonella typhimurium tester strains are widely used for routine testing of unknown chemicals for mutagenic activity. This project is attempting to characterize such factors as the growth kinetics of the strains during the assay, effects of various growth media on the mutagenic response, and the kinetics of the mutagenic response in host-mediated assay systems.

PHS-6040

PROJECT DESCRIPTION

METHODS EMPLOYED: During the course of a standard *Salmonella* plate test bacteria are recovered from the test plates and their growth rates charted to determine lag time and doubling time. The effects of various minimal media formulations and growth media are compared. The relationships between lag time, growth rate, and mutagenic response will be determined for a number of representative chemicals. The intrasanguinous host-mediated assay will be used to determine both organ distribution of the *Salmonella* and organ-specific mutagenic responses of various organ-specific and non-specific carcinogens.

MAJOR FINDINGS AND PROPOSED COURSE: This project has just been initiated and only preliminary work has been performed. A number of formulations of minimal media have been selected and the test protocols are being worked out.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Strains of *Salmonella typhimurium* are widely used for routine testing of mutagens because of their sensitivity and ease of use. A further understanding of the strengths and deficiencies of these strains will enable us to better interpret Salmonella mutagenicity data. Also, it appears possible to make the *Salmonella* tests even more efficient and sensitive than they are now for detection of mutagens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60104-01 LMG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

A Volatile Mutagen From Sodium Azide

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Zeiger	Research Microbiologist	LMG	NIEHS
Other:	J. Guthrie	Microbiologist	LMG	NIEHS

COOPERATING UNITS (if any)
Laboratory of Animal Genetics, NIEHS
Environmental Biology & Chemistry Branch, NIEHS
Biometry Branch, NIEHS

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.05	0.20

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Sodium azide is a base-pair substitution mutagen that is widely used as a positive control chemical for Salmonella typhimurium TA-100 and TA-1535. It was found that when petri dishes containing sodium azide were placed in a 37°C incubator, a volatile mutagen was produced which elevated mutagenic responses on other petri dishes. This volatile mutagen was produced at concentrations as low as 1 μg sodium azide per plate.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The standard *Salmonella* plate test protocol was employed without using a metabolic activation preparation. Sodium azide was used at concentrations of 0, 0.1, 1.0, and 10.0 μg/plate for strains TA-100 and TA-1535. Six plates were prepared for each strain and each concentration of azide. Three plates from each group, randomly selected, were sealed in plastic bags and placed in a non-vented 37°C incubator; the other 3 plates were placed directly in the same incubator. After 2 days incubation, the plate counts of the 0 and low azide plates left unbagged were higher than the corresponding plates in sealed plastic bags.

MAJOR FINDINGS AND PROPOSED COURSE: A volatile mutagen is produced during *Salmonella* plate tests with sodium azide. This mutagen is capable of increasing the numbers of mutants in petri dishes co-incubated with sodium azide plates.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The finding of a volatile mutagen associated with sodium azide indicates the need for caution when using sodium azide as a positive control chemical in a mutagen testing program. Also, this finding has health implications for individuals working with sodium azide as a reagent.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60105-01 LMG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Genetic Control of Mutation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. M. Mason	Staff Fellow	LMG	NIEHS

COOPERATING UNITS (if any)

M. M. Green, Department of Genetics, University of California, Davis, CA

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It has become evident in the last several years that the mutation rate is under genetic control. In bacteria and yeast the frequency of induced mutations can be either increased or decreased by blocking one or another pathway of DNA repair. This project is designed to determine the relationship between DNA repair and mutagenesis in Drosophila melanogaster. Two approaches are being taken: (1) Mutagen-sensitive mutants have been isolated and characterized as to their effects on DNA repair and are being tested for effects on mutation frequency; and (2) mutants which increase the spontaneous mutation frequency (mutators) will be isolated and characterized.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard genetic manipulations utilizing well-characterized mutants and chromosomal aberrations in *Drosophila melanogaster* are employed.

MAJOR FINDINGS AND PROPOSED COURSE: The *X*-linked mutagen-sensitive (*mus*) mutants have been tested for effects on the frequency of spontaneous sex-linked recessive lethals. None of the mutants increases the spontaneous mutation frequency, but two mutants at the same locus (*mus-101*) seem to decrease it. Mutants in the *mus-101* gene are defective in post-replication repair of damaged DNA.

Mutants with known defects in DNA repair have also been tested for effects on nitrogen-mustard-induced recessive lethals and heritable translocations. Again, *mus-101* mutants show a decrease in both of these. Other mutants had no noticeable effect. These mutants are now being examined for their effects on mutations induced by X-rays and alkylating agents.

A screen is being developed which will select for mutators which increase the frequency of spontaneous mutants at the *Adh* locus. No mutants have yet been found using this screen. However, two other mutators have been examined. One of these increases the frequency of recessive lethals 30-fold and somatic recombination 150-fold. The other breaks chromosomes so that terminal deficiencies are recovered. Unlike the aberrations produced by X-rays these broken chromosomes do not appear to be capped by any previously existing telomere. This mutant appears to be able to generate telomeres *de novo*.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study will lead to an understanding of the cellular mechanisms used to regulate the rates of mutation and chromosome breakage.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60106-01 LMG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Cytogenetic Analysis of Mutagen-Sensitive Mutants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. M. Mason	Staff Fellow	LMG	NIEHS

COOPERATING UNITS (if any)

J. B. Boyd, Department of Genetics, University of California, Davis, CA

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mutagen-sensitive mutants are collected in Drosophila melanogaster as a source of mutants defective in DNA repair mechanisms. The mutants are characterized cytogenetically in order to gain a basic understanding of the genetic control of sensitivity to mutagenic agents. The tests used in the initial characterization of these mutants include genetic and cytogenetic mapping, complementation analysis tests for sensitivity to unrelated mutagens, and tests for pleiotropic effects on related functions such as recombination. Some of these mutants may not be defective in DNA repair, but may affect metabolic (in)activation or permeability to chemical mutagens.

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard genetic manipulations utilizing well-characterized X-linked mutants and chromosomal aberrations in *Drosophila melanogaster* are employed. Because the mutagen-sensitive (*mus*) mutants are X-linked the presence of these mutants is monitored by mating *mus* males to attached-X females, treating the progeny with MMS (or other mutagen), and checking the sex ratio of the survivors.

MAJOR FINDINGS AND PROPOSED COURSE: Preliminary characterization of the mutagen-sensitive (*mus*) mutants has been completed. Most of the mutants so far have been isolated because of sensitivity to MMS. These comprise 12 complementation groups which map at random along the X chromosome. Except for two loci, the number of mutants at each locus fits a Poisson distribution with a mean of about 2. This suggests that all, or almost all, X-linked genes controlling sensitivity to MMS have been discovered. Since the X chromosome contains about 20 percent of the genome, this in turn suggests that there are approximately 60 of these MMS-sensitive loci in the Drosophila genome. In addition, there are likely to be genes which control senstivity to other mutagens, but not MMS.

One of the chromosomes isolated as being X-ray sensitive carries *mus* mutants at two different loci. Mutants at one of these loci (*mus-109*) are not X-ray sensitive while a mutant at the second locus (*mus-107*) is not MMS sensitive. If mutants at *mus-107* are X-ray sensitive in the absence of *mus-109* mutants then the *mus-107* locus is unique among known *mus* loci in Drosophila.

Mutants at *mus-102* increase recombination about 20-fold, preferentially in centromic regions. However, it is not clear whether this is an increase in the normal level of euchromatic recombination or whether recombination is induced in heterochromatin where it normally does not occur. To test this possibility recombination will be monitored between two genes which are located in the heterochromatin with no euchromatin between them. It is also possible to monitor recombination between the heterochromatin on the X chromosome and the Y, which is entirely heteochromatic. The observed increase in recombination may be caused by misrepair of spontaneously broken chromosomes.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An understanding of the action of genes controlling mutagen sensitivity is necessary for understanding DNA repair, mutagenesis, recombination and chromosome stability.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60107-01 LMG

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Molecular Cloning and Sequence Analysis of Various Regions of the T4 Genome

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. A. Conkling	Geneticist	LMG	NIEHS
OTHER:	A. Sugino	Visiting Scientist	LMG	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The bacteriophage T4 is a powerful tool in the analysis of basic mechanisms of mutagenesis. The genetics of the rII region have been extensively used and inferences of particular mutagenic pathways can be made. With molecular cloning and DNA sequencing techniques we can now confirm certain inferred pathways. Cloned sequences will also aid in the biochemical analysis of certain T4 functions involved in fidelity and repair. The genome of bacteriophage T4 will be cloned by a strain of phage recombinant DNA techniques. The primary cloning vector will be lambda. The cloned sequences will be pooled and probed for various regions of interest either by DNA:DNA hydridization or by their ability to complement or recombine with T4 phage defective for the desired activity. These regions will then be sequenced or used in biochemical analyses.

PROJECT DESCRIPTION

METHODS EMPLOYED: Purified T4 DNA will be randomly sheared to various lengths by sonication. Treatment with S1 nuclease will then produce blunt ends. Chemically synthesized EcoR1 linkers will be ligated to these ends using T4 DNA ligase. The fragments bearing EcoR1 linkers will be annealed and ligated to vectors and transfected into host cells. The progeny of the transfection should contain random clones of phage T4. Clones of interest will be selected by hybridization, complementation or marker rescue. These clones may then be sub-cloned on the plasmid pBR322. If the linker experiment does not yield the desired fragments we will restrict cytosine-containing T4 DNA. The restriction fragments will then be cloned following the above protocol.

MAJOR FINDINGS AND PROPOSED COURSE: We have had difficulty in obtaining good blunt end ligation of the EcoR1 linkers to the T4 DNA fragments. We have attributed this problem to improperly prepared T4 DNA substrates. We have initial shotguns of cytosine-containing T4 DNA but these have yet to be characterized.

Due to difficulties in cloning the T4 genome, we are developing a technique to sequence regions of the T4 rII genes without first cloning them. This technique will use an end-labled restriction fragment of the rII gene. This fragment will be annealed to T4 DNA and act as a primer for the dideoxy-sequencing method of Sanger.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Much of what we know of basic mechanisms of mutagenesis has been inferred from the genetic system of phage T4. The cloning and sequencing of certain mutants will allow us to verify proposed pathways of mutagenesis by environmental mutagens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60108-01 LMG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Mechanisms of DNA Replication in Prokaryotes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Sugino	Visiting Scientist	LMG	NIEHS
OTHER:	K. Nakayama	Visiting Fellow	LMG	NIEHS

COOPERATING UNITS (if any)
Department of Biochemistry, the University of Chicago, Chicago, Illinois
Department of Bacteriology and Immunology, University of North Carolina, Chapel Hill

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.65	0.65	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

DNA gyrase, which is involved in DNA replication, integrative recombination of phage λ DNA, DNA repair, and transcription, was purified from E. coli, T4-infected E. coli, or B. subtilis. The enzyme consists of the nalidixic acid (oxolinic acid) resistance and novobiocin (coumermycin A1) resistance gene products. In this year, the reaction mechanism of DNA gyrase has been extensively studied in order to determine the role of this enzyme in many important processes in cells. The mechanisms of inhibitory action of novobiocin and nalidixic acid have also been investigated.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Conventional column chromatographies and velocity sedimentation were used for purification of DNA gyrase. To identify the genes for DNA gyrase, resistance markers for nalidixic acid and coumermycin were used. Agarose gel electrophoresis was used for assay of DNA gyrase activities.

MAJOR FINDINGS AND PROPOSED COURSE: DNA gyrase has many enzymatic activities including DNA supercoiling, DNA untwisting, DNA-dependent ATPase, and a DNA breakage reaction in the presence of nalidixic acid and SDS. In this year, the DNA-dependent ATPase intrinsic to DNA gyrase from *E. coli* has been studied to ask how ATP hydrolizing energy is utilized for the DNA twisting reaction. From this study we could conclude that ATP binding is sufficient to introduce a limited number of DNA supertwists. Furthermore, enzyme turnover (in association with the introduction of supercoilings) also requires ATP hydrolysis. We estimated the hydrolysis of one ATP molecule per two supertwists introduced into ColE1 DNA. Novobiocin (or coumermycin) only inhibits ATP-requiring reactions. On the other hand, nalidixic acid (or oxolinic acid) inhibits the reactions involving the nicking-closing event. Nalidixic acid not only inhibits the supercoiling reaction, but also induces DNA substrate breakage in the presence of SDS. The breakage product contains protein(s). We have identified the protein attached to DNA and it is exclusively subunit A of DNA gyrase.

In order to determine the universality of DNA gyrase activities and structure, we have purified the activity from *B. subtilis* and T4-infected *E. coli*. DNA gyrase from T4-infected *E. coli* is resistant to novobiocin but not to nalidixic acid, and we are currently trying to identify the T4 gene which is responsible for the novobiocin resistance of the new DNA gyrase. The DNA gyrase activity of *B. subtilis* is very similar to the *E. coli* DNA gyrase. However, the DNA-breakage reaction of *B. subtilis* DNA gyrase is about 20-fold less active than that of the *E. coli* DNA gyrase. The genes of *B. subtilis* DNA gyrase have been identified; they are *nalA* and *novoA*, located near to the DNA replication origin (*B. subtilis* has two novobiocin genes, *novoA* and *B*).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies help to understand the complexity of DNA replication, particularly the importance of the tertiary structure of DNA, and will provide a corresponding understanding of mutagenic mechanisms.

PUBLICATIONS

Sugino, A., Higgins, N. P., Brown, P. O., Peebles, C. L. and Cozzarelli, N. R.: Energy coupling in DNA gyrase and the mechanism of action of novobiocin. *Proc. Natl. Acad. Sci. USA*, 75: 4838-4842, 1978.

Peebles, C. L., Higgins, N. P., Kreuzer, K. N., Morrison, A., Brown, P. O., Sugino, A. and Cozzarelli, N. R.: Structure and activities of *Escherichia coli* DNA gyrase. *Cold Spring Harbor Symp. Quant. Biol.*, 43: 41-52, 1978.

Sugino, A. and Cozzarelli, N. R.: The intrinsic ATPase of DNA gyrase. J. Biol. Chem., in press. 1979.

Sugino, A., Higgins, N. P. and Cozzarelli, N. R.: *Escherichia coli* DNA gyrase-DNA complex induced by nalidixic acid and sodium dodecyl sulfate - Identification of a protein linked to the end of DNA. Nucleic Acids Res., in press. 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60109-01 LMG

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Mechanism of DNA Replication in Mitochondria from Drosophila Eggs and Embryos

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.75	0.5	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to define the origin of DNA replication in Drosophila mitochondria, mitochondrial DNA treated with restriction enzymes has been cloned in Escherichia coli. Using cloned plasmid DNA containing the DNA replication origin, we currently have been determining the nucleotide sequence. At the same time, an in vitro DNA replication system which resembles the in vivo system has been developed using native mitochondrial DNA and the chimeric DNA.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The standard cloning method using plasmid pBR322 for a cloning vector. The chemical DNA nucleotide sequencing method developed by Maxam and Gilbert. DNA-synthesizing crude extract from isolated mitochondria of *Drosophila* embryos. Agarose and polyacrylamide gel electrophoresis, sucrose density gradient sedimentation, and electromicroscope to analyze the products of *in vitro* DNA replication.

MAJOR FINDINGS AND PROPOSED COURSE: We have succeeded in cloning the Hind III-C fragment of mitochondrial DNA from *Drosophila virilis* embryos. This fragment contains the DNA replication origin and the "A+T-rich" region. Using this cloned DNA segment, we have constructed a detailed physical map of the region around the DNA replication origin and are currently determining the DNA nucleotide sequence. After this sequence is determined, the precise DNA replication origin will be localized using the 6-7 S replication intermediate isolated from replicating mitochondrial DNA (labeled with [r-^{32}P] ATP) and polynucleotide kinase.

At the same time, we have tried to develop an *in vitro* DNA replication system which closely mimics *in vivo* replication using crude extracts from isolated mitochondria. This system is entirely soluble and is able to complement with exogenous protein factors. The products of this system are now being analyzed. If they closely resemble those produced *in vivo*, we will fractionate the activity to individual proteins and reconstitute it to understand the mechanism of DNA replication in mitochondria.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The *in vitro* DNA replication system from mitochondria is a simple model system to purify the protein factor(s) which play a role in DNA fidelity in eukaryotes. This kind of study helps to understand some of the mutation mechanisms in eukaryotes.

PUBLICATIONS

Sugino, A., Shah, D. M. and Langley, C. H.: Structure and function of A+T-rich region in *Drosophila* mitochondrial DNAs. ICN-UCLA Symposium on Molecular and Cellular Biology - Extrachromosomal DNA. *J. Supramol. Structure*, Supplement *3*: 156, 1979.

THE JACKSON LABORATORY - Bar Harbor, Maine
(NIH-N01-ES-4-2156)

TITLE: Development of a System for Detecting Lethal Mutations in Mice

CONTRACTOR'S PROJECT DIRECTOR: Thomas H. Roderick, Ph.D.

PROJECT OFFICER (NIEHS): John W. Drake, Ph.D., Chief, LMG

DATE CONTRACT INITIATED: January 15, 1974

CURRENT ANNUAL LEVEL: $75,000

PROJECT DESCRIPTION

OBJECTIVES: At present no efficient test system exists for detection of point mutations in mammals. The aim of the contract is to develop strains of mice, carrying marked inversions, which may be used in studies of induction of point mutations. Theoretically these tests will be comparable to the system presently used in Drosophila. The prime goal is to induce inversions in as many chromosomes of the mouse as possible and combine them in a number of strains to be used as tester stocks.

METHODS EMPLOYED: The contractor induces chromosome breaks and inversions by means of radiation and/or chemical agents. He mates the treated animals and in the F_1 does cytological analysis of one testis of each individual male. If a significant increase in anaphase bridges appears, this is taken as an indication that the animal may carry an inversion; and he is mated to get offspring for further study. Following cytological and linkage studies to characterize the aberration, a decision is made as to the utility of the inversion and appropriate marker genes are introduced into the stock.

MAJOR FINDINGS AND PROPOSED COURSE: The contractor has induced and recovered 31 inversions. Of these, 19 have been thoroughly characterized for linkage and chromosomal morphology. One of the inversions has been successfully utilized in tests to detect induced recessive lethal mutations. The contract will terminate August 1, 1979.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Recessive lethal mutations are known to exist at high frequencies in human populations. Many environmental compounds are known to be mutagenic and capable of causing both chromosome aberrations and point mutations. With an efficient animal model to study the induction of such mutations, more accurate estimates can be made of the risks such compounds imply to the human population.

LAWRENCE LIVERMORE LABORATORY - Livermore, California
(222Y01-ES-80038)

TITLE: Survey of Cooked Foods for Mutagenic Activities

CONTRACTOR'S PROJECT DIRECTOR: M. L. Mendelsohn, Ph.D.

PROJECT OFFICER (NIEHS): John W. Drake, Ph.D., Chief, LMG

DATE CONTRACT INITIATED: September 22, 1978

CURRENT ANNUAL LEVEL: $400,000

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this contract is to survey the food consumption habits of the general population of the U.S.A., to screen appropriate foods for mutagenic activities using a modified tier approach with particular emphasis on mutagenic activities introduced by food processing procedures (especially cooking), to determine the chemical identities of such mutagens and their mechanisms of formation, to determine their potencies in mammalian systems and to design practical avoidance procedures.

METHODS EMPLOYED: The survey aspects of this program are subcontracted to the Department of Nutrition of the University of California, Berkeley, who also carry out food acquisition and preparation. The initial mutagenicity assay will consist of the Ames battery, supplemented by sister-chromatid exchange and cultured mammalian cell specific-locus mutation assays. Chemical fractionation of complex mixtures in order to isolate mutagenic components will employ a variety of chromatographic methods.

MAJOR FINDINGS AND PROPOSED COURSE: The appearance of mutagenic activity in hamburger meat has been confirmed during the course of cooking under realistic pan-frying conditions (200°C). The efficiency of extraction of the activity has been improved over previously reported methods by the development of an acetone-extraction technique. The activity studied to date is a frameshift mutagen which requires metabolic activation, and initial characterization suggests that it may consist of heterocyclic aromatic amines. Means to obtain food consumption survey information are being explored and developed. Chemical fractionation of cooked meat is now underway. A number of foods and cooking methods will be examined for their effects on mutagenic activities.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The now clearly established presence of mutagenic activities introduced into foods by the process of cooking raises major questions concerning the distribution of such activities in typical North American diets and the risks which they may - or may not - pose to human health. A broad and comprehensive attack is needed, one which will range from survey through analysis to assessment of risk and finally to prevention of genotoxic effects.

RADIAN CORPORATION - Austin, Texas
(NO1-ES-8-2144)

TITLE: Chemical Repository for Mutagenicity Screening

CONTRACTOR'S PROJECT DIRECTOR: L. H. Keith, Ph.D.

PROJECT OFFICER (NIEHS): Douglas B. Walters, Ph.D., Chemist, ECB

DATE CONTRACT INITIATED: September 30, 1978

CURRENT ANNUAL LEVEL: $143,328

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this Contract is to establish a repository for 3000-4000 compounds which are to be screened for mutagenicity. Available physical - chemical and toxicological information is provided on all compounds and chemical analysis is performed when required.

METHODS EMPLOYED: The contractor receives chemicals which are to be tested for mutagenicity by bioassay laboratories under contract. Information is compiled for safety and handling documents as well as complete data sheets on physical - chemical properties, structure, name, toxicology and miscellaneous information. The materials are coded for blind testing, divided into appropriate aliquots, indexed, cross referenced and inventoried into a computerized system. The compounds are sent by a safe, appropriate route to the testing laboratory at a rate of 10-15 samples per month including controls. An estimated 10% of test compounds will be analyzed for trace chemical impurities.

MAJOR FINDINGS AND PROPOSED COURSE: A total of 206 test compounds and 24 controls have been selected for FY 79 testing. To date 160 test compounds and 23 controls have been received by the repository. All chemicals are stored in the contractor's Hazardous Materials Laboratory. Catalog file data (chemical properties, etc.) are compiled as compounds are placed in inventory status. Work is nearing completion on implementing a computerized inventory and data-handling system. Satisfactory survivability tests have been completed and approximately 60 aliquots have been shipped to 3 test labs. A summary of operating procedures for the Hazardous Materials Laboratory has been written. One chemical has been subjected to satisfactory chemical analysis.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The development of a comprehensive testing system for mutagenesis (as well as other toxicity testing) requires a repository which can be computerized for effectiveness and efficiency and which is designed around a specially designed containment laboratory for handling hazardous materials. The laboratory also must provide for routine chemical assay as well as sophisticated, complete, chemical trace impurity analysis. These requirements are necessary to support *in vitro* and *in vivo* testing.

1. CASE WESTERN RESERVE UNIVERSITY - Cleveland, Ohio
(N01-ES-9-2136)

2. EG&G MASON INSTITUTE - Rockville, Maryland
(N01-ES-9-2137)

3. SRI INTERNATIONAL - Menlo Park, California
(N01-ES-9-0001)

TITLE: Microbial Mutagenesis Testing

CONTRACTOR'S PROJECT DIRECTOR:
1. William Speck, M.D.
2. Steven Haworth, Ph.D.
3. Vincent F. Simmon, Ph.D.

PROJECT OFFICER (NIEHS): Errol Zeiger, Ph.D., Research Microbiologist
Environmental Mutagenesis Test Development
Program, LMG

DATE CONTRACT INITIATED:
1. December 22, 1978
2. December 29, 1978
3. February 1, 1979

CURRENT ANNUAL LEVEL:
1. $44,184
2. $38,538
3. $63,172

PROJECT DESCRIPTION

OBJECTIVES: The purpose of these contracts is to test a total of 225 environmental and commercial chemicals for mutagenicity using *Salmonella typhimurium* tester strains in 3 laboratories. Substances found mutagenic in *Salmonella* will be selected for testing in mammalian and Drosophila systems.

METHODS EMPLOYED: *Salmonella typhimurium* strains TA-98, TA-100, TA-1535, and TA-1537 are being used to test for mutagenicity using a modification of the Ames *Salmonella* microsome assay. All chemicals will be incubated with the tester strains in suspension prior to addition of soft agar and plating for detection of induced mutants. Exogenous metabolic activation will be provided by liver S-9 preparations from Arochlor 1254-induced Sprague-Dawley rats and Syrian Hamsters. All chemicals will be tested blind at 5 doses, in triplicate, in each *Salmonella* strain. Also, all chemicals will be retested at least 2 weeks following the first test. Results will be entered on data forms and transferred to a computerized data base system.

MAJOR FINDINGS AND PROPOSED COURSE: The testing aspects of these contracts have just gotten underway and complete results are not yet available. It is expected, however, that testing will proceed rapidly.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These contracts will allow the NIEHS to rapidly screen large numbers of chemicals for mutagenicity in a relatively short time and at relatively low cost. Mutagenicity in this system correlates strongly with carcinogenicity and heritable mutations in rodents. The chemicals found mutagenic, therefore, will be given the highest priority for chronic toxicological and carcinogenesis testing by the National Toxicology Program.

LABORATORY OF ORGAN FUNCTION AND TOXICOLOGY

LABORATORY OF ORGAN FUNCTION AND TOXICOLOGY

A new laboratory, "Laboratory of Organ Function and Toxicology (LOFT)" is being created from LET. This laboratory consists of researchers from a variety of scientific disciplines: toxicology, physiology, pharmacology, pathology, biochemistry and endocrinology. This laboratory (1) provides a central research focus at the NIEHS for ongoing investigation of the effects of environmental agents on renal, gastrointestinal, and hepatic function and proposed investigations on the cardiovascular and immune systems; (2) plans and conducts studies to elucidate the mechanisms by which environmental agents alter normal functions of these organ systems including the role of metabolism, endocrine factors, and toxicant-receptor interactions; (3) plans and conducts studies to identify sensitive indicators of organ specific toxicity useful in the early detection and prediction of toxicity in experimental animals and humans; and (4) collabrates with epidemiologists in developing methods for extrapolation of data from experimental animal models to estimation of human risks from exposure to environmental agents.

HEPATIC AND ENDOCRINE

The research of this group is concerned with various aspects of hepatotoxicity emphasizing the more subtle alterations in liver function following exposure to environmental agents. Areas studied include enzymology, endocrinology, pathology and pharmacology in adult and developing systems. A multidisciplinary approach is utilized to 1) identify sensitive biochemical indicators of hepatotoxicity that might have clinical value; 2) elucidate mechanisms of hepatotoxicity; 3) understand the regulation of hepatic protein synthesis including the role of the hypothalamic-pituitary-gonadal axis; and 4) characterize the role of endocrine action in the regulation of hepatic function in control and treated animals including the role of hormone receptors and toxicant-receptor interactions. Biochemical, physiological and morphological changes are studied in relation to the binding, metabolism, and clearance of hormones and foreign chemicals.

Research efforts are in progress to elicidate the mechanisms of perinatal imprinting of hepatic metabolism by hormones and hormonally-active xenobiotics such as the PCBs. Treatment of newborn rats with DES or TP produces no change in the prepubertal activities of several hepatic enzymes (5α-reductase, 16α-hydroxylase, histidase, UDP glucuronyltransferase, ethylmorphine demethylase, monamine oxidase, etc.). However, the subsequent adult animals exhibit masculinized or feminized levels of enzyme activity and these effects appear to be permanent and irreversible. A series of experiments utilizing ectopic pituitaries and hypophysectomized rats suggest that imprinting of enzyme activity is mediated through the pituitary-hypothalamic axis during a critical period of early development. Although, imprinting of hepatic enzyme synthesis might have a common mechanism, different regulatory models can be constructed for different groups of enzymes. Hepatic protein profiles are being mapped by 2-D electrophoresis in an attempt to further understand the sex differentiation of hepatic function in control and treated animals. Further experiments show that sensitivity to the toxic effects (liver and testes) of cadmium are permanently altered qualitatively and quantitatively by newborn exposure to hormonally-active xenobiotics.

Certain organs such as uterus, vagina and mammary gland are stimulated by estrogen. The presence of receptor is presumed obligatory to a direct estrogenic effect on cellular machinery. Estrogen has been shown to modulate various aspects of mammalian liver function. Moreover, adverse side effects of estrogenic components of oral contraceptives on the liver are receiving increasing attention. Certain of these estrogen-induced changes could reflect a direct liver-hormone interaction dependent upon the presence of estrogen receptors in the liver cells. Our studies demonstrate that liver cytosol fractions from both male and female rats contain estrogen-binding components possessing criteria assigned to receptor proteins. These criteria include a finite binding capacity together with a high affinity and binding specificity for estrogens. Interestingly male liver contains an additional estrogen-binding protein distinct from the receptor. The physiological significance of this protein is under investigation. These binding proteins appear to be under pituitary-hypothalamic control. In contrast to uterus, two conformational forms of estrogen-receptor complexes are evident in both male and female liver nuclei. The presence of receptors indicates that the liver is a target organ for estrogens. The study of hepatic estrogen-receptor interactions and the consequences of this estrogen action is clearly of importance in determining the impact of estrogenically-active chemicals on liver function.

RENAL AND INTRACELLULAR

Scientists in this workgroup study basic biochemical and physiological mechanisms through which environmental agents exert their toxic effects in vivo. The goal is to apply this information to designing rapid and effective test procedures for predicting toxicity in man with specific emphasis on the kidney.

Principal efforts are oriented towards investigation of the mechanisms involved in producing the earliest signs of subcellular damage by both quantitative electron microscopy, biochemical, and physiological measurements. Particular emphasis is placed on understanding the mechanisms by which environmentally important chemicals interact with these processes to effect toxicity in human populations. Specific studies include investigations concerning the mechanisms by which agents such as cadmium produce renal damage and low molecular weight proteinurias. Previous studies from this group have shown that renal uptake of cadmium bound to cadmium metallothionein (Cd-MT) is mediated via the proximal tubule cell lysosome system with subsequent degradation of the metalloprotein to release Cd^{+2} ion. The mechanism by which this process causes marked necrosis of proximal tubule cells is under current investigation. A second area of intense interest involves the mechanisms behind toxicant damage to mitochondrial structure-function relationships and the role of this phenomenon in important mitochondria-mediated clinical entities such as Reye's Syndrome. Toxic agents like arsenic have been shown to alter mitochondrial membrane structure and synthesis with attendant compromise of biochemical processes dependent on the physical integrity of this organelle. Further studies are in progress to delineate the molecular basis of these structural changes and their relationship to biochemical mitochondrial dysfunction.

The third area of effort involves combined use of ultrastructural and morphometric and biochemical techniques to identify early effects of heavy metals and other environmental agents on mammalian tissues so that early warning tests for predicting sub-oxic human responses to such agents may be developed. These studies include determining the correlation between quantitative subcellular morphologic observations and biochemical alterations in tissues of animals chronically exposed to low levels of toxic agents for various periods. In current studies, researchers are examining the toxicity of arsenic, cadmium, lead, methyl and inorganic mercury and free fatty acids. Results of their experiments indicate that specific morphological and biochemical response profiles can be established which differentiate various metals and other agents in our multi-element environment. Studies are currently in progress to use these profiles to develop test procedures that will permit identification of preclinical exposure to specific environmental substances in human populations by measurement of circulating metabolites such as porphyrins.

GASTROINTESTINAL

This workgroup focuses on the organ-specific responses to toxic insults and also, on the basic functions of specific organ systems. The workgroup is concerned, in particular, with the function and toxicology of the gastrointestinal tract. Currently, the research of this workgroup includes basic studies designed to examine 1) metabolism and transport in the developing small intestine, 2) the structure and metabolism and absorption of isolated intestinal crypt and tip cells, and 3) processes which are characteristic of specific intestinal functions and/or specific cellular membranes.

The toxicological aspects of the gastrointestinal research include acute and chronic exposure of animals to environmentally-important bioactive compounds both pre- and postnatally as well as in vitro studies of the potentially toxic effects of these compounds. Indicators of toxic responses include alterations to enzyme development patterns, isozyme profiles and specific cell-type enzyme indications or inhibitions as well as tumor formations. These approaches provide for a varied research program that allows for the determiation of alterations in structure and function. The basic studies are designed to complement the toxicological studies so that when alterations do occur a cellular and subcellular site of action can be ascertained and studies to elucidate the mechanisms of the effect implemented. In the future emphasis will be given to the uniue transport functions and cellular differentiation of this organ. The possible effects of the interactions of ingested substances both in natural and metabolized forms on these processes will be considered.

In the past, perinatal development studies of the small intestine have dealt primarily with hydrolytic enzymes, the brush border, and the absorption of specific nutrients. Our emphasis has been on the development of the metabolic energy producing processes required for the active transport of nutrients and also the effect of various bioactive environmental compounds on the normal developmental patterns of this target organ.

Many of our results have long-range significance in providing a better understanding of the development of normal intestinal function, the normal morphology and metabolism of intestinal cells and the unique mitochondrial and energy metabolism of intestinal tissue. Other observations include results which may have toxicological and clinical utility for the medical community. Examples of the latter include our studies on the responses to treatments with 1,2-dimethylhydrazine, 2,3,7,8-tetrachlorodibenzo-p-dioxin and arsenic.

COLLABORATIVE RESEARCH

NIH CONTRACTS

Laboratory scientists are Principal Investigators in a wide variety of research contract efforts. These collaborative efforts are logical extensions of the Laboratory's intramural programs concerned with reproductive, developmental, and target organ toxicities.

Drs. Lucier and McLachlan are collaborating with the Wisconsin Alumni Research Foundation to search for the transplacental toxicity of selected environmental chemicals. This contract emphasizes reproductive tract function and transplacental carcinogenesis and fulfills a critical need to identify chemicals or classes of chemicals that might adversely affect reproductive tract function following gestational exposures. Chemicals currently under study include zeranol, several PCB and PBB isomers, DDE, polycyclic hydrocarbons, and a series of mycotoxins.

Dr. Fowler supervises a contract with the University of Washington to investigate the potential carcinogenic actions of petroleum hydrocarbons using selected estuarine organisms as the model. This research is exploring the relationship between tumor induction and metabolism of the chemical carcinogen, benzo(a)pyrene. The exposure system has been developed, the microsomal enzyme assay system optimized, and the tumor biopsy procedure refined.

The Hepatic and Endocrine workgroup (Dr. Lucier) and Brigham Young University are attempting to identify clinically-useful biochemical indicators of developmental toxicity. The approach is to first investigate the ontogeny of key enzyme systems following treatment with known teratogens.

COLLABORATIVE RESEARCH WITH ACADEMIC COMMUNITY AND GOVERNMENTAL AGENCIES

There are numerous examples of collaborative research projects at the local, national and international level. Laboratory scientists have acknowledged the resources and expertise concentrated in nearby universities. Moreover, we have not hesitated to establish the collaborative efforts necessary to develop quality intramural programs with scientists throughout the world, including government, academia, and private industry.

Dr. Fowler and NOAA scientists are investigating the physiological effects of arsenic, cadmium, and copper on marine shellfish. This effort is directed at elucidating the mechanisms by which commercially-important marine shellfish concentrate heavy metals. An unusual cadmium-binding protein has been isolated from shellfish which appears to play an important role in the bioaccumulation of this toxicant by commercially important species. The toxic actions of cadmium and copper on gill respiration are being examined in relation to induction of this protein.

Dr. Lucier is studying the role of hormone receptors in xenobiotic-induced alterations of hormone responsiveness with the UNC Department of Biochemistry. This project should yield information important to understanding the mechanism of action of endocrine-mediated hepatotoxicity. In a related project, Drs. Lucier, Lamartiniere, and Fiegelson (Roosevelt Hospital, New York) are attempting to utilize hepatic histidase as a marker indicative of perinatal imprinting of hepatic metabolism. Dr. Schiller is working with Dr. Robert Jeffcoat (RTI) on the metabolism of p-nitrophenol in isolated intestinal cells in relation to redox state.

NATIONAL AND INTERNATIONAL PROGRAMS: SYMPOSIA ORGANIZED/COMMITTEE APPOINTMENTS, ETC.

Another important indicator of peer approval and scientific relevance of current Laboratory programs is the frequency that LET & LOFT scientists organize and participate in "state of the art" symposia and are asked to serve on various committees attempting to provide meainingful direction in environmental health research. Descriptions of representative examples of Laboratory involvement in this regard are given below.

Dr. Schiller is the NIEHS representative to the NIH Digestive Disease Coordinating Committee as well as Nutrition Coordinating Committee. She is also chairman of the committee on In Vitro Research and Human Values of the Tissue Culture Association. Dr. Schiller has been conducting experiments with Dr. Hans Falk (NIEHS) and Dr. B. Paccagnella of the University of Padova, Verona, Italy on the in vitro effects of trifluorotoluene on cell respiration. A symposium sponsored, in part, by the World Health Organization on the health effects of these compounds is being planned. Dr. Schiller has also organized a symposium in the target organ series, "Target Organ Toxicity: Intestines (Intestinal Function)."

Dr. Fowler has been active in national and international programs dealing with metal toxicity. He has most recently been responsible for organizing a International Conference on Cadimum. In addition, he has acted as a temporary advisor to the World Health Organization for development of an arsenic criteria document. At present, he is a member of the Society of Toxicology Technical Committee and the Working Group on the Carcinogenic Risk of Chemicals to Humans for the International Agency for Research on Cancer (IARC).

Dr. Lucier is a member of the Toxicology Forum Panel, which is making recommendations regarding protocol development for in utero toxicity testing. He has been an invited participant and session chairman at numerous conferences and workshops involving developmental pharmacology and toxicology including the International Congress of Pharmacology in Paris where he chaired a session on "Perinatal Pharmacokinetics." Dr. Lucier is Program Director for EPA Pass-Through funds for energy-related research on organ specific toxicity and he has been asked to write several review articles on organ specific metabolism and toxicity.

INFORMATION EXCHANGE

Communication of basic and applied information vital to environmental health problems is aided by establishing mechanisms for information exchange and by assuming editorial responsibilities. Dr. Lucier is the editor of the NIEHS journal, Environmental Health Perspectives and on the Editorial Board of the Journal of Applied Biochemistry.

TRAINING PROGRAMS

Environmental health is a new and demanding research area that is undergoing rapid change and growth. Consequently, there is a growing need for training to ensure adequate numbers of qualified and dedicated researchers in environmental health research. The Laboratory of Environmental Toxicology and Laboratory of Organ Function and Toxicology recognizes this need and our scientists are encouraged to participate in a wide variety of training programs, including adjunct appointments at nearby universities, supervision of graduate student research, development of graduate courses in environmental health, and participation in the Fogarty Visiting Program. Several LET & LOFT scientists have adjunct appointments with nearby universitties. Dr. Lucier is Adjunct Associate Professor in the Biochemistry Department of the UNC School of Medicine; Dr. Fowler is Adjunct Assistant Professor in the Pathology Department of UNC School of Medicine; Dr. Schiller is Adjunct Assistant Professor of Biochemistry in the UNC School of Medicine and is also on the faculty of the W. Alton Jones Cell Science Center, Lake Placid, New York.

Drs. Schiller and Lucier were involved in organizing and teaching a graduate course in Biochemical Toxicology at the University of North Carolina in the Biochemistry Department. Dr. Lucier also lectured to the Biochemical Toxicology course offered by North Carolina State University.

Laboratory scientists have also been active in the training of graduate students. Drs. Schiller and Lucier are supervising the graduate research of several students in the UNC Biochemistry Department. Postdoctoral training is strongly supported by LET & LOFT, and our commitment to these programs involves the support of Visiting Fellows and Visiting Associates.

Dr. Lucier is participating in the Toxicology Training Program being developed by the University of North Carolina.

Drs. Lucier, McLachlan, Fowler, Schiller, Korach, and Harris have lectured at UNC and/or Duke in areas of their research expertise.

LABORATORY OF PHARMACOKINETICS

LABORATORY OF PHARMACOKINETICS
Summary Statement

The Laboratory of Pharmacokinetics has the responsibility for planning and developing a focus at NIEHS for pharmacokinetic studies and collaborative efforts applying pharmacokinetic principles to enable an understanding of where and why chemicals accumulate in living organisms and how these uptake, storage, and elimination processes are regulated or can be altered. To carry out this mission, Laboratory scientists plan and conduct studies to develop pharmacokinetic models for specific classes of chemicals in various species, and to develop strategies for extrapolating chemical behavior and toxicity from one species to another and to man. They plan and conduct studies on use of pharmacokinetic data to design better treatments for toxicities due to persistent chemicals, to evaluate body burdens of environmental chemicals, and to assess effects of different rates of exposure to environmental chemicals on toxicological outcomes. Scientists evaluate the role of metabolism in chemical accumulations across various species. They establish and maintain liaison with various groups in and out of government using pharmacokinetic approaches in toxicological evaluations. Finally, Laboratory scientists design and conduct studies on the disposition and pharmacokinetics of selected chemicals under study in the National Toxicology Program. These studies will facilitate the choice of dose for subchronic toxicology testing, and will be used to explain variations in toxicity observed with species, sex, dose or subtle changes in chemical structure.

There are currently no formal sections in the Laboratory. Rather, research is conducted in separate but complementary areas, using approaches ranging from whole animal studies to *in vitro* systems, but all aimed at meeting Laboratory objectives.

Whole Animal Disposition and Metabolism

A primary research focus in the Laboratory continued on measuring whole animal disposition of chemical pollutants with particular emphasis given to the role of metabolism and macromolecular binding in explaining difference in disposition and toxicity between structurally related chemicals.

Detailed studies on the *in vivo* metabolism of mono and dichlorinated biphenyls, and a series of symmetrical hexachlorobiphenyls showed that the major metabolites of the PCBs are mono- and di-hydroxylated derivatives which are excreted as glucuronide conjugates. It was established that the presence of two adjacent unsubstituted carbon atoms are required for a rapid rate of metabolism and subsequent decrease in body burden. Metabolism occurs primarily through formation of arene oxide intermediates, and further, there was shown to be a correlation between the rate of metabolism to intermediates and covalent binding to hepatic RNA, DNA and protein.

In further work aimed at refining flow-limited pharmacokinetic models for interspecies extrapolation of pharmacokinetic profiles, it was shown under a contract with the University of Arizona that the patterns of metabolism and disposition of PCBs in dog and monkey are similar to patterns seen in rodents, i.e., the presence of adjacent unsubstituted carbon atoms is necessary for appreciable metabolism and excretion to occur, and the major metabolites are hydroxylated products. In general, biological half-life of a PCB proceeds as monkey $>$ rat $>$ dog. The data from these higher species is currently being fitted to our models as an important next step toward our objective of trying to extrapolate pharmacokinetic data from lower animals to man.

An investigation was undertaken of the metabolism, disposition and excretion of two halogenated alkyl phosphate fire retardants in an attempt to explain marked differences in toxicity in compounds closely related in chemical structure. Tris (tris[2,3-dibromopropyl]phosphate) is highly mutagenic and carcinogenic while Fyrol FR-2 (tris[1,3-dichloroisopropyl]phosphate) is weakly mutagenic and noncarcinogenic. It was found that although the metabolism, biological half-life and excretion patterns were similar between the two chemicals, Tris treatment of rats resulted in 10 fold greater alkylation of DNA than did Fyrol treatment.

In another study under contract, the metabolism and disposition of several environmental chemicals was studied, including azobenzene, Tris and benzidine derivatives. These studies were designed to provide a better understanding of factors which influence the rates of absorption, distribution and excretion of chemicals of interest to intramural scientists and/or the National Toxicology Program, and to provide the data necessary to an estimation of biological half-lives, times to steady-state, and possible chronic toxicity of the compounds.

A number of chemicals, including several antioxidants, are known to have anti-carcinogenic effects. Recently initiated research in our Laboratory is aimed at understanding whether these chemicals exert their anti-carcinogenic effects through alteration of the pharmacokinetics of affected carcinogens. The effects of butylated hydroxy anisole (BHA) on the metabolism and excretion of benzo(a)pyrene(BP) were studied in rats. Preliminary findings from this study suggest that the anti-carcinogenic effects of BHA may be in part due to a decrease in enterohepatic recycling of BP metabolites, and to a shift in the pattern of hydroxylated metabolites.

Isolated Organ Pharmacokinetics

This group focuses on studies of uptake, storage, metabolism, and release of pollutants and model compounds by isolated perfused organs with present emphasis on the lung. The isolated perfused rodent lung (IPL) system, along with lung slice studies, allows examination of the pharmacokinetics of chemicals localizing in the lungs. One current objective is to evaluate the role of the lungs in the whole body clearance of aromatic chemicals including the role of conjugative processes as determinants of pulmonary clearance of chemicals subject to conjugation. α-Naphthol was selected as a model compound for these studies in the IPL. Metabolism of α-naphthol by the lung was rapid and concentration dependent with formation of the sulfate conjugate being the major metabolic pathway.

Relation of the Pharmacokinetics Program to the Mission of NIEHS

In a broad sense, the NIEHS mission is to detect, prevent, and modify environmentally induced disease through identifying and understanding environmental stressors and their combinations. Before we can prevent or modify the toxic effects of environmental chemical agents, we must be able to determine how and at what rates chemicals enter the body, and how and at what rates the body handles the chemicals in terms of distribution, storage, metabolism, and excretion. From studies on the rates and mechanisms of chemical disposition, we can better understand how and where chemicals exert their toxic effects, and how and why there are qualitative and quantitative differences among organs and tissues and between species in pharmacologic-toxicologic effects. Hopefully, from such information, we will be able to extrapolate experimental findings from animal test systems across species and ultimately to man.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80009-08 LPK

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Pharmacokinetic and Pharmacodynamic Functions of the Lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Alan G. E. Wilson	Visiting Associate	LPK NIEHS
Other:	Ada C. Kung	Visiting Fellow	LPK NIEHS

COOPERATING UNITS (if any)

Inhalation Toxicology Section, Environmental Biology Branch

LAB/BRANCH
Laboratory of Pharmacokinetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 2	PROFESSIONAL: 1	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less – underline keywords)

The objective of this research is to evaluate the pharmacokinetic importance of the lung in the uptake, metabolism, storage and release of endogenous and exogenous chemicals. Pharmacokinetic data is correlated with toxicological, physiological and biochemical changes induced during the accumulation of chemicals by the lung. These studies make considerable use of the isolated perfused lung (IPL) preparation. The IPL permits examination of the pharmacokinetics of chemicals reaching the lung both from the circulation and inhaled air. Pharmacokinetic models are constructed from the data and related to pulmonary damage resulting from exposure to environmental chemicals. The role of the lung in the whole body distribution of chemicals will be assessed and incorporated into pharmacokinetic models.

PHS-6040

PROJECT DESCRIPTION

PART I. INVESTIGATION OF THE PHARMACOKINETICS OF ACCUMULATION, TOXIFICATION AND DETOXIFICATION OF CHEMICALS AND POLLUTANTS BY THE LUNG.

METHODS EMPLOYED: The lung perfusion apparatus consists of an artificial thorax, a recirculating or "single-pass" perfusate supply, and an air supply. The lungs are ventilated with 95% air-5% CO_2 by applying an alternating negative pressure to the artificial thorax. The circulatory system was designed with a perfusate reservoir directly above the lungs to which the labeled chemicals were added. Perfusate samples are removed and analyzed following passage through the lungs. This apparatus permits studies of the rates of uptake and efflux of chemicals by the lung. The perfused lung has been modified to permit studies of the metabolism and retention of inhaled chemicals. The trachea is connected directly to either a vapor or an aerosol generator. Appearance of the chemical and its metabolites in the perfusate and expired air are continuously monitored. The system is designed to permit study of the effect of exposure to the inhaled chemical on the physiological function of the lung.

Lung slices, incubated in a Krebs-Ringer bicarbonate buffer containing 5 mM glucose are also employed to obtain information on the uptake and metabolism of chemicals by lung tissue.

Studies on the metabolic conjugation of chemicals by the lung employed a recirculating IPL system. Perfusate samples are analyzed for metabolites using chromatographic and enzymic hydrolysis methods.

MAJOR FINDINGS AND PROPOSED COURSE: α-Naphthol was selected as a model compound to study the role of the lungs in the metabolic conjugation of chemicals. α-Naphthol was rapidly metabolized by the rabbit isolated perfused lung (IPL) in a concentration dependent manner. A single exponential decay of α-Naphthol from the perfusate was observed. Clearance was dependent upon initial dose for concentrations above 0.1 μM. Below this concentration linear kinetic conditions appear to exist. At this concentration approximately 60% of the original α-Naphthol was conjugated in a first-pass effect. Metabolism was predominantly to the sulfate conjugate, with minor, but significant amounts due to glucuronide conjugation.

The proposed course is as follows: 1) To complete the pharmacokinetic profile for α-Naphthol in the rabbit IPL and compare with rat IPL. Evaluate role of lung in total body elimination of α-Naphthol.

2) To investigate metabolism of other aromatic phenols and hydrocarbons and evaluate the role of conjugative processes as determinants of pulmonary clearance of environmental chemicals.

3) To study the effect of modifiers on the uptake and metabolism of inhaled compounds, e.g., particulates.

4) To elucidate the mechanism(s) and differences between the pulmonary metabolism and kinetics of inhaled and blood-borne chemicals.

PART II. STUDIES ON THE EFFECT OF ENVIRONMENTAL AGENTS ON THE PHARMACOKINETICS OF UPTAKE AND METABOLISM OF ENDOGENOUS (A) AND EXOGENOUS (B) CHEMICALS BY THE LUNG.

METHODS EMPLOYED: Male Spraque-Dawley rats and male and female New Zealand rabbits were exposed to vaporized morpholine at 250 ppm, 6 hr/day, for periods up to 8 weeks. Lungs were then removed and prepared for perfusion or lung slices as described in Part I.

A) ENDOGENOUS CHEMICALS (5-Hydroxytryptamine)

MAJOR FINDINGS AND PROPOSED COURSE: 5-Hydroxytryptamine (5HT) is known to be removed from the pulmonary circulation by a sodium dependent unidirectional transport system. 5-HT is subsequently metabolized by intracellular monoamine (MAO) to 5-hydroxyindole acetic acid which rapidly effluxes from the lung. Measurement of the pulmonary clearance of 6-HT has been suggested to be a valuable index in the assessment of lung damage resulting from exposure to chemicals. We have used this system to evaluate the pulmonary damage suggested to occur as a result of inhalation exposure of animals to the industrial solvent morpholine. Exposure of rats to morpholine (LD$_{10}$) over a 5-week period produced a significant decrease in the pulmonary clearance of 5-HT. This effect was due to an inhibition of the metabolism of 5-HT by MAO rather than impairment of the uptake of 5-HT by the lung. This was verified by the finding that morpholine was a potent MAO inhibitor, displaying competitive inhibition kinetics for a variety of MAO substrates. As part of this study the substrate specificity of pulmonary MAO's was characterized. This particular study has been completed.

B) EXOGENOUS CHEMICALS (Imipramine)

MAJOR FINDINGS AND PROPOSED COURSE

We have previously developed pharmacokinetic models to describe the mechanisms responsible for the accumulation and persistence of basic amines in the lung. These studies suggested that the alveolar macrophage may play an important role in the storage and persistence of these chemicals by the lung. We have been using these pharmacokinetic models to assess possible pulmonary damage resulting from exposure to environmental agents. Exposure of rabbits to morpholine was shown to impair the pulmonary accumulation and persistence of imipramine. This was evidenced as a reduction in the size of a specific pharmacokinetic pool. This pool is responsible for the persistence of imipramine in the lung and its morphological location is most probably the alveolar macrophage. The reduction in the size of this pharmacokinetic pool was dependent on the length of exposure to morpholine. Alveolar macrophages from morpholine exposed rabbits were shown to have impaired biochemical functions and reduced phagocytic ability. These effects may be related to the effect seen on imipramine pharmacokinetics. This is presently being investigated by the use of cultured alveolar macrophages.

The proposed course is as follows: 1) To extend our studies on the effect of environmental chemicals on the pulmonary pharmacokinetics of endogenous and exogenous model compounds.

2) To examine the uptake and metabolism of environmental agents and relate their disposition to the induction of pulmonary damage.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The lung is an important site for the uptake and detoxification of a number of important endogenous compounds. Understanding the significance of this pulmonary accumulation and metabolism is essential to an understanding of lung function and the effect of exposure to environmental pollutants on the lung. Elucidation of the mechanisms of pulmonary uptake and metabolism of chemicals may permit early prediction of pulmonary damage, such as the reduced pulmonary clearance of 5-HT seen in oxygen toxicity. In addition, such knowledge may allow possible prediction of toxic interactions between endogenous and exogenous substances.

PUBLICATIONS

Kung, H. C. and Wilson, A. G. E.: Characterization of rat pulmonary monoamine oxidases. Life Sci. 24: (No. 5) 425-432, 1979.

Wilson, A. G. E., Picket, R. D., Eling, T. E., and Anderson, M. W. Studies on the persistence of basic amines in the rabbit lung. Drug Metab. Disp. (In Press).

Kung, H. C. and Wilson, A. G. E. Effect of morpholine on the pulmonary clearance of 5-hydroxytryptamine. Toxicol. Appl. Pharmacol. (In Press).

Wilson, A. G. E., Sar, M. and Stumpf, W. E. Imipramine uptake by the isolated perfused lung. An autoradiographic study with pharmacokinetic considerations. Eur. J. Drug. Metab. and Pharmacokinetics. (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 35003-01 LPK

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Pharmacokinetic Parameters in Carcinogenesis: Their Alteration by Anti-carcinogenic Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marshall W. Anderson	Mathematician	LPK NIEHS
	Alan G. E. Wilson	Visiting Associate	LPK NIEHS
Other:	Medhi Boroujerdi	Visiting Fellow	LPK NIEHS
	Ada C. Kung	Visiting Fellow	LPK NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Pharmacokinetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 3	PROFESSIONAL: 1.5	OTHER: 1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less – underline k

Numerous studies suggest that the in vivo metabolism of benzo(a)pyrene (BP) and other carcinogens is altered when animals are pretreated with anti-carcinogenic agents such as butylated hydroxy anisole (BHA). Based on these observations, we have initiated studies to determine the type and amount of in vivo metabolic alteration and DNA binding of BP in animals pretreated with BHA. At present, the profiles of the glucuronide and sulfate conjugates in the bile have been determined in control and BHA treated animals. No sulfate conjugates were detectable in control or treated animals. Glucuronide conjugation accounted for 30% of the dose in control and treated animals - BHA pretreatment altered the profile of the glucuronide metabolites. The amount of 6-hydroxy BP was decreased in BHA treated animals and the amount of 4,5-dihydrodiol BP was concomitantly increased. This could have implications as to the anti-carcinogenic action of BHA since 6-hydroxy-BP has been suggested to be a carcinogenic metabolite of BP. Recycled metabolites of BP were also decreased by BHA pretreatment.

PROJECT DESCRIPTION

METHODS EMPLOYED: Initially, the bile-cannulated rat will be used as the model to study in vivo metabolism of carcinogens in control animals and animals pretreated with various anti-carcinogenic agents. Benzo(a)pyrene (BP) and BHA are our initial choices of carcinogen and inhibitor. After incubating bile samples with the appropriate deconjugating enzymes, the profiles of the glucuronide and sulfate conjugates were determined by high pressure liquid chromatography (HPLC). Techniques are being developed to analyze for glutathione conjugates. An HPLC analysis is also being developed to separate glucuronide, sulfate, and glutathione conjugates. Urine and blood samples will also be analyzed for BP metabolites. The binding of BP metabolites to DNA and the identification of DNA adducts is being investigated concurrent with the metabolic studies.

MAJOR FINDINGS AND PROPOSED COURSE: After an iv administration of 1.0 moles/Kg of ^{3}H-BP to rats, eighty percent of the dose is excreted in the bile in six hours, both in control and treated animals. Only conjugated metabolites appear in the bile. Very few, if any, sulfate conjugates were detected in control or treated animals. Approximately 30% of the dose was excreted as glucuronide conjugates in control animals. The profile of the glucuronide conjugates in control animals is summarized as follows: 1) Phenols account for 2.5% of the administered dose. 2) The 4,5-dihydrodiol accounted for 1.5% of the dose and the 7,8-dihydrodiol was detected as a small but significant amount, 0.8%, of the dose while no 9,10-dihydrodiol was detected. 3) Quinones were detected in the bile samples after incubation with β-glucuronidase and accounted for 9% of the dose. Our results suggest that these quinones were formed from 6-hydroxy BP. Thus, at least 9% of the administered dose of BP is metabolized to 6-hydroxy BP. 4) Glucuronide conjugates of metabolites more polar than the 9,10-dihydrodiol are present in the bile. They account for 10% of the dose. At present their structure is unknown, but they probably represent metabolites that have been metabolized at least twice (recycled) by the mixed-function oxidases. 5) No hydrolysis products, i.e. tetrols, of the diol epoxides were detected.

When incorporated into the diet at 0.75% and 3.0%, BHA produced dose-dependent alterations in the metabolic profile of the glucuronide conjugates. A comparison between the glucuronide conjugates in control and BHA pretreated (0.75%) animals is summarized as follows: 1) The amounts of phenols formed are unaltered. 2) In BHA pretreated animals, the amount of 4,5-dihydrodiol increased from 1.5 to 7% of the dose and the 7,8-dihydrodiol decreased from 0.7 to 0.4% of the dose. 3) The total amount of quinones decreased from 9 to 5.5% of the dose in BHA pretreated animals. In particular, the BP-3,6-dione decreased from 6 to 2.5% of the dose. 4) The recycled metabolites decreased from 10 to 8% of the dose in 0.75% BHA treated animals and to 1.6% of the dose in 3.0% BHA treated animals.

The in vivo binding of BP to liver and lung DNA was examined. The total amount of radioactivity covalently bound to DNA was 100 times higher in liver than in lung. However, no BP metabolite-nucleoside adduct was detected in liver but an adduct was detected in lung and accounted for 15% of the DNA bound radioactivity in lung. The nature of this adduct is unknown at present. BHA pretreatment had no effect on the DNA bound radioactivity in either liver or lung.

Several conclusions from our studies suggest possible mechanisms for the anticarcinogenic action of BHA. The decrease in the recycled metabolites of BP after BHA pretreatment could result from the increased levels of glutathione S-transferase induced by BHA. Some of the recycled metabolites could be ultimate carcinogens. The diol epoxides are recycled metabolites and certain of them are proposed as ultimate carcinogens in vivo. Although we did not detect any diol epoxides, they might be present in small amounts. The free radicals generated from the auto-oxidation products of 6-hydroxy BP have also been implicated as ultimate carcinogens. Our results clearly show that BHA pretreatment decreases the amount of 6-hydroxy BP formed in vivo. The proposed course is as follows: 1) We plan to study the in vivo metabolism and DNA binding of BP in animals pretreated with various inducers of cytochrome P-450 and animals pretreated with inducers plus BHA. 2) We will determine in vivo DNA binding under conditions where BHA pretreatment has inhibited BP carcinogenesis. 3) Techniques will be developed to analyze for glutathione conjugates of BP metabolites and metabolites of other polycyclic aromatic hydrocarbons. 4) Dose-response studies will be done of the in vivo metabolism and DNA binding of BP.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many chemicals inhibit carcinogenesis when administered prior to or simultaneously with the carcinogen. To understand the mechanism of action of these anticarcinogenic agents would permit the rational design of more potent anticarcinogenic agents. In addition, these studies on the inhibition of carcinogenesis should provide some insight into the carcinogenic action of BP and similar environmental pollutants.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 35004-01 LPK

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Metabolism and Disposition of Halogenated Alkyl Phosphates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Hazel B. Matthews	Research Chemist	LPK NIEHS
	Nydia Morales	Visiting Fellow	LPK NIEHS
	Amin Nomeir	Visiting Fellow	LPK NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Pharmacokinetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.4	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The metabolism, disposition, and excretion of two halogenated alkyl phosphate fire-retardants have been studied in the male rat. Each of these compounds, tris (2,3-dibromopropyl) phosphate (Tris) and tris (1,3-dichloroisopropyl) phosphate (Fyrol FR-2), are rapidly absorbed, metabolized and excreted. The major metabolites are the products of dealkylation reactions which are mediated by both microsomal and soluble enzymes. Excretion is in the urine, feces and as exhaled CO_2. There is some evidence that more Tris than Fyrol is covalently bound to the DNA of kidney when animals are exposed to similar doses in vivo.

PROJECT DESCRIPTION

METHODS EMPLOYED: The metabolism, distribution and excretion of two halogenated alkyl phosphates have been studied in the male rat following oral, dermal or iv administration. The compounds studied were tris (2,3-dibromopropyl) phosphate (Tris) and tris (1,3-dichloroisopropyl) phosphate (Fyrol FR-2). Each was radiolabeled with carbon-14. The metabolism of each compound was studied both *in vivo* and *in vitro*. Metabolites were isolated by extraction with organic solvents and purified by thin-layer and high-performance liquid chromatography. Identification of metabolites was achieved by cochromatography with chemically synthetized metabolites. Tissue distribution was studied by sampling at various time points and oxidizing the samples to $^{14}CO_2$ in a biological material oxidizer. Excretion was followed by collecting samples of urine, feces and exhaled CO_2. Quantitation of samples was by liquid scintillation counting.

MAJOR FINDINGS: 1) Each of these compounds is readily absorbed from both the skin and intestines and distribution is similar regardless of the route of exposure.

2) The major mechanisms of metabolism involve dealkylations of the phosphate. The dealkylated products undergo various degrees of further metabolism and may be metabolized all the way to CO_2.

3) These compounds have short biological half-lives and are excreted primarily in the urine with lesser amounts being eliminated in the feces or metabolized to CO_2 and exhaled.

4) Trace amounts of each of these compounds are metabolized to reactive intermediates which alkylate subcellular macromolecules.

5) The major differences relating to structure are observed in the alkylation of DNA in the kidney where treatment with Tris results in approximately 10 fold greater DNA alkylation than results from Fyrol treatment.

PROPOSED COURSE:

These studies will be completed, the results prepared for publication and this project will be terminated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITITUTE: The halogenated alkyl phosphates studied in this project have been or are currently used as fire-retardants in fabrics and flexible foam upholstery. Both Tris and Fyrol were at one time used in children's pajamas. Tris has been shown to be mutagenic in the Ames bioassay system and carcinogenic to laboratory animals; however Fyrol is an order of magnitude less mutagenic in the Ames bioassay system and extensive testing has yet to show that Fyrol is carcinogenic to laboratory animals. Therefore, studies of these two compounds offer an opportunity to relate structure to activity in the mediation of a toxic effect by closely related compounds. The results of these studies will facilitate an understanding of the factors which result in toxicity and will provide information which should aid in the recognition of other potentially toxic compounds or facilitate the design of less toxic compounds which may be synthesized in the future.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80014-06 LPK

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Metabolism of Polychlorinated Biphenyls

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Hazel B. Matthews	Research Chemist	LPK NIEHS
	Shigeru Kato	Visiting Fellow	LPK NIEHS
	James D. McKinney	Research Chemist	ECB NIEHS

COOPERATING UNITS (if any)

Environmental Biology Branch

LAB/BRANCH
Laboratory of Pharmacokinetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Reserach Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.9	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Most polychlorinated biphenyls (PCBs) are readily absorbed from the intestines of higher animals, but metabolism to more polar compounds is a prerequisite to PCB excretion. The retention of PCBs is inversely proportional to the rate of metabolism. It is the position and not necessarily the degree of chlorination that determines the rate of PCB metabolism. Two adjacent, unsubstituted carbon atoms are required for a rapid rate of PCB metabolism because their presence facilitates oxidative metabolism, probably via an arene oxide intermediate. However, PCBs without two adjacent unsubstituted carbon atoms may be metabolized via an arene oxide intermediate by shifting a chlorine, using the NIH shift, or by directly inserting a hydroxyl group into the meta position. The major metabolites of PCBs are mono- and di-hydroxylated derivatives which are excreted as glucuronide conjugates.

PROJECT DESCRIPTION

METHODS EMPLOYED: The studies have utilized ^{14}C-labeled PCBs and in vivo metabolism by the rat to obtain the necessary metabolites. The metabolites were isolated by various extraction techniques, purified by thin-layer and column chromatography, and identified by mass spectral and nuclear magnetic resonance analysis. A second approach which is being utilized in the study of the very slowly metabolized PCBs is the synthesis of the anticipated metabolites. These "synthetic metabolites" are then used to identify the authentic metabolites by cochromatography, comparison mass spectrometry, and isotope dilution.

MAJOR FINDINGS AND PROPOSED COURSE: 1) The major metabolites of the PCBs are mono- and di-hydroxylated PCBs, and there is a possibility of an arene oxide intermediate. Metabolism appears to be a prerequisite to PCB excretion. Two adjacent, unsubstituted carbon atoms are necessary for an appreciable rate of metabolism of the PCBs.

2) The major metabolites of 4-chlorobiphenyl have been isolated and identified as: 4-hydroxy-4'-chlorobiphenyl; 3-hydroxy-4'-chlorobiphenyl; 3,4-dihydroxy-4'-chlorobiphenyl; and 3-hydroxy-4-methoxy-4'-chlorobiphenyl. These metabolites account for approximately 60, 7, 23, and 3 percent, respectively, of the radioactivity extracted from the excreta of treated rats.

3) The major metabolite (90%) of 4,4'-dichlorobiphenyl has been isolated and identified as 4-hydroxy-3,4'-dichlorobiphenyl. Traces of the 3-hydroxy-4,4'-dichlorobiphenyl have also been isolated.

4) The metabolites of 2,4,5,2',5'-pentachlorobiphenyl have been described and are 3-hydroxy-2,4,5,4',5'-pentachlorobiphenyl, 3,4-dihydro-3,4-dihydroxy- and 3,4-dihydroxy-pentachlorobiphenyl. Only approximately 13% of this compound is excreted as the parent compound; 60% of the radioactivity excreted was in the form of the monohydroxy; and about 25% was in the form of the dihydrodiol and dihydroxy metabolites.

5) 2,3,5,2',3',5'-Hexachlorobiphenyl was slowly metabolized and excreted as 4 different metabolites, 2,3,5,2',3',5'-hexachloro-4'-hydroxybiphenyl; 2,3,5,2',3',5'-hexachloro-6'-hydroxybiphenyl; 2,3,5,2',4',5'-hexachloro-3'-hydroxybiphenyl and 2,3,5,2',5'-pentachloro-4'-hydroxybiphenyl which accounted for 1.03, 0.09, 0.38 and 0.003% of the total dose, respectively. Excretion of unmetabolized 2,3,5,2',3',5'-hexachlorobiphenyl accounted for 2.96% of the total dose. Greater than 90% of the total dose was not excreted.

6) 2,3,6,2',3',6'-Hexachlorobiphenyl was metabolized and excreted quite rapidly relative to the other hexachlorobiphenyls studied with 95% of the total dose excreted in seven days. Only a single metabolite of this PCB was observed, 2,3,6,2',3',6'-hexachloro-4-hydroxybiphenyl, and that metabolite accounted for 86% of the material excreted with the remainder being parent compound.

7) Less than 10% of a dose of 2,4,5,2',4',5'-hexachlorobiphenyl was excreted during the first seven days following its administration to adult male rats. The metabolites isolated and identified were the 3'- and 6'-hydroxy derivatives of the parent compound, 2,4,5,2',3',5'-hexachloro-4'-hydroxybiphenyl and the 3'- and 4'-hydroxy derivatives of 2,4,5,2',5'-pentachlorobiphenyl; the metabolites accounted for 0.30, 0.064, 0.20, 0.098 and 0.003% of the total dose, respectively. Approximately 5% of the total dose was excreted as parent compound.

8) A total of 13.46% of a dose of 2,4,6,2',4',6'-hexachlorobiphenyl was excreted during the first seven days after iv administration. Most of this material represented a single metabolite, 2,4,6,2',4',6'-hexachloro-3'-hydroxybiphenyl, and only 1.75% was parent compound.

9) These studies have established that the presence of two adjacent, unsubstituted carbon atoms facilitates the metabolism of PCBs via an arene oxide intermediate and that in the absence of adjacent, unsubstituted carbon atoms, the greater number of equivalent open meta positions, the greater the metabolism, probably by direct insertion of a hydroxyl group.

The proposed course of this work is to complete metabolic studies of two tetrachlorobiphenyls and terminate the project.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The polychlorinated biphenyls are ubiquitous environmental contaminants, and knowledge of their biotransformation and the identification of their metabolites is essential to our understanding of their importance as potential hazards to man and his environment. We have evidence that arene oxides are formed in the metabolism of PCBs. Arene oxides have been implicated in a number of undesirable biological reactions including teratogenesis, carcinogenesis, and tissue necrosis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80016-06 LPK

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Pharmacokinetics of Chlorinated Xenobiotics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Hazel B. Matthews	Research Chemist	LPK NIEHS
Other:	Nydia M. Morales	Visiting Fellow	LPK NIEHS
	Daniel B. Tuey	Staff Fellow	BB NIEHS
	Shigeru Kato	Visiting Fellow	LPK NIEHS
	Wayne A. Colburn	Staff Fellow	LPK NIEHS

COOPERATING UNITS (if any)

Biometry Branch, NIEHS; Chemical Engineering Section, BEIB, DRS, NIH

LAB/BRANCH
Laboratory of Pharmacokinetics

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 2.9	PROFESSIONAL: 1.5	OTHER: 1.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

Previous work in this laboratory has described the absorption and tissue distribution of halogenated hydrocarbons and the importance of metabolism to the excretion of these compounds. The results of current studies demonstrat that adjacent unsubstituted carbon atoms in the meta-para positions of polychlorinated biphenyls (PCBs) facilitate their metabolism via arene oxide intermediates and results in greater binding to subcellular macromolecules. Other current work demonstrates that the adipose tissue is not a well mixed pharmacokinetic compartment and that this fact may result in inaccurate extrapolation of disposition data from acute to chronic studies.

PROJECT DESCRIPTION

METHODS EMPLOYED: The distribution, accumulation, and excretion of eight ^{14}C-labeled PCBs, Kepone, and a polybrominated biphenyl (PBB) were studied after iv and oral administration to rats. Four of the PCBs were also studied in the mouse. The major tissues and excreta were sampled at times varying from a few minutes to several weeks post-treatment. Distribution and excretion were studied under conditions of normal feeding and starvation. Analyses were facilitated by the use of a biological materials' oxidizer and liquid scintillation counting. All of the data were subjected to further analysis by computer.

The major subcellular macromolecules, DNA, RNA and protein, were isolated at three time points following the administration of ^{14}C-labeled PCBs, and the amount of binding was related to the substitution pattern of the PCB administered. A combination of ^{14}C and ^{3}H labeled 2,4,5,2',4',5'-hexachlorobiphenyl was used to determine if the fat is a well mixed pharmacokinetic compartment.

MAJOR FINDINGS AND PROPOSED COURSE: 1) The role of metabolic activation in the binding of PCBs to subcellular macromolecules has been studied in vivo by comparing the relative amounts of binding of 2,4,5,2',4',5'-hexachlorobiphenyl, a slowly metabolized PCB, with that of 2,3,6,2',3',6'-hexachlorobiphenyl, a rapidly metabolized PCB. The covalent binding of 2,3,6,2',3',6'-hexachlorobiphenyl to hepatic RNA, DNA and protein exceeded the covalent binding of 2,4,5,2',4',5'-hexachlorobiphenyl by as much as 36, 41 and 26 fold, respectively.

2) Studies with tritium and carbon-14 labeled 2,4,5,2',4',5'-hexachlorobiphenyl have been conducted which demonstrate that the adipose tissue is not a well mixed pharmacokinetic compartment. Thus, it was demonstrated that the second of two doses of 2,4,5,2',4',5'-hexachlorobiphenyl administered was excreted more rapidly than the first. This observation is referred to as the "last-in, first-out phenomenon."

3) In the course of studying the biliary excretion of halogenated aromatic chemicals, a simple technique was devised to extend the period of surgical anesthesia in laboratory rats. The method utilizes the recommended intraperitoneal dose of pentobarbital plus the oral administration of a second dose of pentobarbital diluted with water to extend the period of surgical anesthesia by up to four fold.

The proposed course is as follows: 1) The disposition of several other types of lipophilic halogenated hydrocarbons will be studied in order to test the pharmacokinetic models which have been devised to predict the disposition of PCBs.

2) Further studies will be conducted to determine the relationship of the biliary excretion and enterohepatic circulation of halogenated xenobiotics with their toxicity and disposition.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Halogenated xenobiotics are among the most persistent of our environmental contaminants. These compounds are known to cause a variety of biological disorders in man as well as being at least partially responsible for the declining numbers of certain species of wildlife. Accumulation of high concentrations in the tissues of animals exposed to relatively low doses is a characteristic of certain chlorinated xenobiotics. Yet many of these compounds are used in our current methods of food production, disease control, and certain industrial processes. Thus, we need to be able to predict what will be the disposition of these compounds in animals and man. Understanding the pharmacokinetics of halogenated hydrocarbons in different species will help in this prediction or extrapolation of animal data to man and may lead to more specific modes of treatment including better ways to accelerate removal of such compounds from the body.

PUBLICATIONS

Matthews, H., Fries, G., Gardner, A., Garthoff, L. Goldstein, J., Ku, Y., and Moore, J.: Metabolism and biochemical toxicity of PCBs and PBBs. Environ. Hlth. Persp. 24: 147-155, 1978.

Matthews, H. B.: A simple method for extending the period of surgical anesthesia in rats. Lab. Animal Sci. 28: 720-722, 1978.

Matthews, H. B. and Kato, S.: The metabolism and disposition of halogenated aromatics. Ann. N. Y. Acad. Sci. In press.

Morales, N. M., Tuey, D. B., Colburn, W. A. and Matthews, H. B.: Pharmacokinetics of multiple oral doses of selected polychlorinated biphenyls in mice. Toxicol. Appl. Pharmacol. In press.

Colburn, W. A. and Matthews, H. B.: Pharmacokinetics of chronic toxicity tests: The last-in first-out phenomenon. Toxicol. Appl. Pharmacol. In press.

Morales, N. M. and Matthews, H. B.: The role of metabolic activation in the binding of polychlorinated biphenyls to macromolecules, *in vivo*. Chem. Biol. Interact. In press.

University of Arizona
Tucson, Arizona
NIH-N01-ES-2111

TITLE: "Extrapolation of PCB Disposition"

CONTRACTOR'S PROJECT DIRECTOR: I. Glenn Sipes, Ph.D.

PROJECT OFFICER (NIEHS): H. B. Matthews, Ph.D., Research Chemist, LPK

DATE CONTRACT INITIATED: April 15, 1977

CURRENT ANNUAL LEVEL: $69,000

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project is to obtain information on the disposition of polychlorinated biphenyls (PCBs) in dogs and monkeys and to use this knowledge in the further development of a pharmacokinetic model which will predict for a number of species the distribution accumulation, and excretion of PCBs and other chlorinated xenobiotics after different dose levels and routes of exposure.

METHODS EMPLOYED: The distribution, accumulation, metabolism, and excretion of ^{14}C-labeled PCBs (4,4'-dichloro-, 2,4,5,2',4',5'-hexachloro-2,3,6,2',3',6'-hexachloro-biphenyl) will be studied in Beagle dog and Fasciculous monkey. All major tissue and excreta (urine and feces) are sampled at various times after administration of the PCB. Biliary studies are also to be conducted to determine the necessary pharmacokinetic parameters needed for modeling. Flow rate to the major organs will be determined in the monkey by means of a microsphere technique.

MAJOR FINDINGS AND PROPOSED COURSE: 1) The half-life of a PCB in the dog or the monkey is inversely proportional to the rate of metabolism of that PCB.

2) The dog is better able to metabolize PCBs having unsubstituted meta positions than is the monkey or the rat, but the dog very slowly metabolizes PCBs in which all the meta positions are substituted.

3) Chlorination in the para position greatly inhibits PCB metabolism by the monkey, but not by the dog.

4) PCBs with adjacent unsubstituted meta and para positions are readily metabolized by all species tested.

5) The adipose tissue and skin serve as the major tissue depots for unmetabolized PCBs in all species studied.

6) Analysis of the tissues and excreta indicate that PCB metabolites have short half-lives in all species studied.

7) The route of PCB excretion varies with molecular weight and species. The importance of excretion in feces increases with increasing molecular weight and the rat and dog excrete a larger portion of the metabolites in feces than does the monkey.

8) The major metabolites of these PCBs are hydroxylated products and the importance of the position of hydroxylation varies with species.

PROPOSED COURSE: The stipulations of this contract have now been fulfilled and the contract will be terminated. The data obtained from the contract is quite good and there is an ongoing effort to use this data in the construction of a physiological pharmacokinetic model which will allow the extrapolation of the disposition of PCBs to several species.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Chlorinated xenobiotics are among the most persistent of our environmental contaminants. These compounds are known to cause a variety of biological disorders in man as well as being at least partially responsible for the declining numbers of certain species of wildlife. Accumulation of high concentrations in the tissues of animals exposed to relatively low doses is a characteristic of certain chlorinated xenobiotics. Yet many of these compounds are used in our current methods of food production, disease control, and certain industrial processes. Thus, we need to be able to predict what will be the disposition of these compounds in animals and man. Understanding the pharmacokinetics of PCBs in different species will help in this prediction or extrapolation of animal data to man.

Virginia Commonwealth University
Richmond, Virginia
N01-ES-6-2117

TITLE: "A Study of the Pharmacokinetics and Toxicity of Kepone in Humans and Laboratory Animals"

CONTRACTOR'S PROJECT DIRECTOR: Philip S. Guzelian, M.D.

PROJECT OFFICER (NIEHS): H. B. Matthews, Ph.D., Research Chemist, LPK

DATE CONTRACT INITIATED: December 15, 1975

CURRENT ANNUAL LEVEL: $34,997

PROJECT DESCRIPTION

OBJECTIVES: The recent discovery of accidental chronic exposure of a small community to Kepone^{R} has presented many problems relating to immediate toxicity and unknown long-term consequences. The goal of this proposal is to conduct a rapid, orderly, and systematic investigation of the absorption, distribution, biotransformation, and excretion of Kepone in humans and animals to aid in understanding its toxicity and to provide a basis for rational therapy.

METHODS EMPLOYED: 1) Human Investigations. Initial clinical findings will be expanded to include tabulation of complete historical, epidemiologic, and physical findings in 25-30 symptomatic patients. Routine clinical lab tests will be done. Blood, saliva, and excreta will be analyzed for Kepone and metabolites. 2) Analytical Methods. Various types of solvent extractions will be used to remove Kepone and metabolites from biological samples, followed by TLC and GLC analysis. 3) Lab Animal Investigations. The relative rates and extent of Kepone excretion in urine, bile, and feces will be compared in different species by the usual methodologies.

MAJOR FINDINGS AND PROPOSED COURSE: Industrial workers exposed to the organochlorine pesticide, chlordecone (Kepone), had signs of toxicity in several organs. The extent of toxicity was proportional to the levels of this chemical in the tissues. In 22 patients, chlordecone was eliminated slowly from blood (half-time of 165 ± 27 days -- mean ± S.E.M.) and fat (half-time of 125 days with a range of 97 to 177), chiefly in the stool. Output of chlordecone in bile was 10 to 20 times greater than in stool, suggesting that chlordecone is reabsorbed in the intestine. Cholestyramine, an anion-exchange resin that binds chlordecone, increased its fecal excretion by seven times. In a five-month trial, cholestyramine significantly accelerated elimination of chlordecone from blood, with a half life of 80 ± 4 days (S.E.M) ($P < 0.005$) and fat (half-life of 64 days, with a range of 52 to 85) ($P < 0.05$) when compared to subjects not treated with the resin. Cholestyramine offers a practical means for detoxification of persons exposed to chlordecone and possibly to other lipophilic toxins. Cholestyramine has also been used with some success to treat human intoxication with chlordane.

PROPOSED COURSE: 1) The investigators will continue to monitor patients for the full course of the contract to record the progress of the symptoms of kepone intoxication and the general health of each exposed individual.

2) The metabolism of Kepone will be studied in monkeys.

3) An effort will be made to investigate the enterohepatic circulation of other lipophilic xenobiotics and to evaluate a series of drugs which might act to interrupt the cycle of enterohepatic circulation and facilitate the excretion of these compounds.

4) The cholestyramine therapy will be used to treat patients intoxicated with other persistent lipophilic xenobiotics as the opportunity arises.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A recent discovery of accident industrial poisoning of workers with the pesticide Kepone and the possibility of contamination of the air, water, and sewage of a small Virginia community with this chlorinated hydrocarbon has created a need to rapidly develop biologic information regarding this agent. The proposed interdisciplinary studies will investigate the toxic and pharmacokinetic properties of Kepone using a combined approach of *in vivo* and *in vitro* systems including man. A well-conducted analysis of this problem may not only yield valuable information on Kepone but may set up a protocol for the investigation of similar incidents of environmental contamination which may occur in the future with other compounds.

PUBLICATIONS

Cohn, W. J., Boylan, J. J., Blanke, R. V., Farris, M. W., Howell, J. R. and Guzelian P. S.: Treatment of chlordecone toxicity with cholestyra-mine: Results of a controlled clinical trial. New Eng. J. Med. 298:243-248, 1978.

Egle, J. L., Fernandez, S. B., Guzelian, P. S., and Borzelleca, J. F.: Distribution and excretion of chlordecone in the rat. Drug Metab. Dist. 6: 91-95, 1978.

Blanke, R. V., Fariss, M. W., Guzelian, P. S., Paterson, A. R. and Smith, D. E.: Identification of a reduced form of chlordecone in human stools. Bull. Environ. Contam. Toxicol. 20:782-785, 1978.

Skalsky, H. L., Fariss, M. W., Blanke, R. V., and Guzelian, P. S.: The role of plasma protems in the transport and distribution of chlordecone and other polyhalogenated hydrocarbons. Anal. N. Y. Acad. Sci. *In press*.

University of Oregon Health Sciences Center
School of Medicine
Department of Pharmacology
N01-ES-7-2126

TITLE: "Pharmacokinetics of Xenobiotics"

PROJECT DIRECTOR: Robert K. Lynn, Ph.D.

PROJECT OFFICER (NIEHS): H. B. Matthews, Ph.D., Research Chemist, LPK

DATE CONTRACT INITIATED: September 30, 1977

CURRENT ANUAL LEVEL: $157,790

PROJECT DESCRIPTION

OBJECTIVES: The objective of this contract is to provide information on the metabolism, distribution and excretion of selected xenobiotics which are of particular interest to the National Toxicology Program or scientists in the intramural program at the NIEHS. These studies are designed to provide a better understanding of those factors which determine the rates of absorption, distribution and excretion of xenobiotics and to provide the data necessary to an estimation of the biological half-lives, times to steady-state and possible chronic toxicity of the compounds studied.

METHODS EMPLOYED: These studies will be conducted in intact animals and will utilize ^{14}C-labeled compounds or established analytical techniques to determine the degree of absorption, major tissue depots, clearance rates, degree of metabolism and rates and routes of excretion. To achieve this a number of animals will be treated similarly, sacrificed in a serial manner, and the major tissues and daily excreta of each animal will be sampled to determine the content of the compounds of interest. The relative amounts of parent compound and metabolites will be determined at selected time points by extraction with organic solvents and various types of chromatographic analysis.

MAJOR FINDINGS: 1) The metabolism of azobenzene has been studied in the rabbit and the rat, and several metabolites have been identified.

2) It has been demonstrated that exposure to light results in photochemical isomerization of azobenzene.

3) The disposition of tris (1,3-dichloroisopropyl) phosphate has been studied in the laboratory rat, and one metabolite has been identified.

4) The metabolism of benzidine derived dyes back to the carcinogenic precursors has been studied in dogs.

PROPOSED COURSE: 1) An effort will be made to quantitate the amount of benzidine, dimethoxybenzidine and dimethylbenzidine excreted in the urine of dogs receiving known quantities of these compounds. Then the amounts of these and other known carcinogens derived from these molecules will be determined in dogs receiving known quantities of dyes which are synthetic derivatives of these molecules. These results will be used to estimate the amount of the various dyes which are biologically converted to the carcinogenic precursors.

2) An effort will be made to modify the above procedure for use with humans known to be exposed to benzidine derived dyes.

3) Additional compounds or groups of compounds will be studied as requested by the NTP or personnel in the NIEHS intramural program.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is the goal of much biomedical research, the NTP and the NIEHS to determine the significance of human exposure to a variety of toxic xenobiotics. A finite amount of data on the metabolism and disposition of toxic xenobiotics is essential to the proper design of chronic studies of such compounds. Furthermore, data obtained from carefully planned and excuted studies of the metabolism and disposition of toxic xenobiotics can be used to more accurately relate laboratory observations to man. It is the role of this contract to provide disposition and kinetic data which will complement studies of toxic xenobiotics under the NTP or in the NIEHS intramural program.

Arizona Board of Regents
University of Arizona
Tucson, Arizona 85724
N01-ES-8-2130

TITLE: "Pharmacokinetics of Xenobiotics"

PROJECT DIRECTOR: I. Glenn Sipes, Ph.D.

PROJECT OFFICER (NIEHS): H. B. Matthews, Ph.D., Research Chemist, LPK

DATE CONTRACT INITIATED: September 15, 1978

CURRENT ANNUAL LEVEL: $143,436

PROJECT DESCRIPTION

OBJECTIVES: The objective of this contract is to provide information on the metabolism, distribution and excretion of selected xenobiotics which are of particular interest to the National Toxicology Program or intramural scientists at the NIEHS. These studies are designed to provide a better understanding of those factors which determine the rates of absorption, distribution and excretion of xenobiotics and to provide the data necessary to an estimation of the biological half-lives, times to steady-state and possible chronic toxicity of the compounds studied.

METHODS EMPLOYED: These studies will be conducted in intact animals and will utilize ^{14}C-labeled compounds or established analytical techniques to determine the degree of absorption, major tissue depots, clearance rates degree of metabolism and rates and routes of excretion. To achieve this a number of animals will be treated similarly, sacrificed in a serial manner and the major tissues and daily excreta of each animal will be sampled to determine the content of the compounds of interest and metabolites. The relative amounts of parent compound and metabolites will be determined at selected time points by extraction with organic solvents and various types of chromatographic analysis.

MAJOR FINDINGS: This is a relatively new contract, the contractor has not yet been asked to study a particular xenobiotic and the current major effort is to devise a mechanism to extrapolate rates of xenobiotic metabolism from one species to another. Some promising preliminary results have been obtained.

PROPOSED COURSE: The present work will be continued for some time to come. However, as soon as studies of particular xenobiotics are requested by the NTP or individuals at the NIEHS the major effort will be to determine the disposition of these compounds. It is the goal of much biomedical research, the NTP, and the NIEHS to determine the significance of human exposure to a variety of toxic xenobiotics.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is the goal of much biomedical research, the NTP, and the NIEHS to determine the significance of human exposure to a variety of toxic xenobiotics. A finite amount of data on the metabolism and disposition of toxic xenobiotics is essential to the proper design of chronic studies on such compounds. Furthermore, data obtained from carefully planned and executed studies of the metabolism and disposition of toxic xenobiotics can be used to more accurately relate laboratory observations to man. It is the role of this contract to provide disposition and kinetic data which will complement studies of toxic xenobiotics which will be done under the NTP or in the NIEHS intramural program.

LABORATORY OF PHARMACOLOGY

LABORATORY OF PHARMACOLOGY
Summary Statement

The Laboratory of Pharmacology initiates and carries out research programs that are primarily concerned with (1) mechanisms by which environmental pollutants and effectors cause toxicity, (2) the development of better test systems for detecting and evaluating early toxic effects of chemicals occurring as environmental contaminants, and (3) extrapolating from animal test systems across species and to man. The Laboratory is organized into two formal sections, the Toxication-Detoxication Section and the Marine Pharmacology and Biomedicine Section.

A. Toxication-Detoxication Section

This program area is concerned with the role of chemical metabolism in the mediation of toxicity such as tissue damage. Metabolic processes leading to more toxic products (activation) may be at least partially counteracted by metabolic processes which form less toxic products (deactivation). This balance of toxication and detoxication pathways may be at least partially responsible for the target organ toxicity associated with specific classes of xenobiotics and may be upset by previous or concomitant exposure to pollutants or drugs, by disease states, or by hormone changes. Some of these activation and deactivation enzymes are also dependent upon animal age, sex, and species.

Chemical-metabolizing enzyme systems have been and are being characterized in tissues that serve as interfaces with the environment and its pollutant load (skin, intestine, and lung), in liver which serves as the major tissue for the biotransformation for most organic xenobiotics, and in some steroidogenic tissues necessary for maintaining homeostasis and reproduction capability (adrenal, testis, and ovary). Characterization of the enzymes in these cases includes substrate specificity, inhibitor effects, ability to be induced or stimulated by previous exposure to environmental contaminants, and separation and purification of individual constituents of multicomponent systems.

B. Marine Pharmacology and Biomedicine Section

This program area conducts studies on the uptake, distribution, metabolism, and excretion of pollutants by various marine species, and the role of metabolism in the storage and the chemical form of the accumulated xenobiotics in these species. The major emphasis is on how, why, and where marine species accumulate pollutants which have potential for harm to man and whether mixtures of pollutants may lead to accumulation of more toxic forms or higher levels of pollutants than single chemical exposure. Effect of water temperature on metabolic, storage, and excretion processes is being studied. The pharmacokinetics of pollutant distribution in selected marine species is also being determined and compared with mammals (with the Laboratory of Pharmacokinetics). Particular emphasis is being placed on studying formation and further metabolism of chemically reactive metabolites (e.g., arene oxides from polycyclic hydrocarbons which are constituents of crude oil). Special importance is also given to the cytochrome P-450 containing MFO system and other hydrolytic and conjugating pathways, including effects of pre-exposure to environmental contaminants on these enzyme systems. Where biologically significant induction is observed, the induced system is characterized in considerable detail. Since most marine species have lesser abilities than mammalian species to oxidize chemicals, the nonoxidative (e.g., conjugating) enzyme systems are being studied in several tissues of marine species. The Section is also involved in developing a national program (NIH focus) in aquatic pharmacology and physiology as directly applicable to human health problems (e.g., models for human disease and sources of toxic materials, such as carcinogens and mutagens for man).

Personnel

Dr. J. R. Fouts (Chief, Laboratory of Pharmacology) spent FY '79 on a foreign study assignment in the Departments of Pathology and Toxicology, Institute of Toxicology, Zurich, Switzerland. Dr. J. R. Bend was Acting Chief, Laboratory of Pharmacology, during Dr. Fouts' absence. In June, Dr. Pritchard transferred his program from NIEHS to the satellite laboratory at the C. V. Whitney Marine Laboratory, University of Florida, St. Augustine. Additions to the Laboratory of Pharmacology were: Biological Laboratory Technician -- Patrick Pitts (with Dr. Pritchard); Visiting Scientist -- Boris Yagen (with Dr. Bend); Visiting Associate -- Louise Ball (with Dr. Bend); Intergovernmental Personnel Act Fellow -- Michael Franklin (with Dr. Fouts); Visiting Fellows -- Anthony Almeida (with Dr. Pritchard), Jane Croft (with Dr. Philpot) and Ken Jones (with Dr. Fouts). Individuals leaving the Laboratory of Pharmacology included Ms. Julie Angerman and Ms. Barbara Stetson.

Other Activities

Dr. J. R. Fouts: Adjunct Professor, Dept. Pharmacology, School of Medicine, University of North Carolina - Chapel Hill; Adjunct Professor, Dept. Entomology, School of Life Sciences, North Carolina State University, Raleigh; Visiting Professor, Depts. Pathology and Toxicology, University of Zurich, Switzerland; member, editorial boards of Molecular Pharmacology, Chemico-Biological Interactions, Xenobiotica, Cancer Research, Journal of Toxicology and Environmental Health, and Associate Editor, Pharmacological Reviews. Liaison member for ASPET on Scientific Committee on Problems of the Environment of the International Council of Scientific Unions.

Dr. J. R. Bend: Adjunct Associate Professor, Dept. of Entomology, North Carolina State University, Raleigh; Member, Editorial Advisory Board for Drug Metabolism and Disposition; Visiting Scientist -- C. V. Whitney Marine Laboratory, University of Florida, St. Augustine; Trustee, Mount Desert Island

Biological Laboratory, Salsbury Cove, Maine; member, Committee on Environmental Pharmacology, American Society for Pharmacology and Experimental Therapeutics; Associate Managing Editor (U.S.A.) for Chemico-Biological Interactions; Associate Editor, Reviews in Biochemical Toxicology; invited participant, symposium on "Metabolism and Disposition of Pesticides and Other Chemicals in Aquatic Organisms," 176th Meeting of the American Chemical Society; session chairman, symposium on "Host Defense Mechanisms in the Respiratory Tract" (EPA); presented invited seminars at the Dept. of Pharmacology, University of Minnesota, Department of Biochemistry, McMaster Medical School and the EPA Water Quality Control Laboratory, Duluth; invited speaker at workshop on "PCB's in the Marine Environment," organized by FDA and the Mid-Atlantic Fishery Management Council; organizer and moderator NIH Science Writers Seminar on "Toxication or Detoxification of Environmental Chemicals"; co-organizer of symposia on "Aquatic Animals as Models in Biomedical Research" and "Comparative Pharmacology in Aquatic Species," on behalf of Committee on Environmental Pharmacology, ASPET.

Dr. R. M. Philpot: Adjunct Associate Professor, Department of Entomology, North Carolina State University, Raleigh; Associate Managing Editor (U.S.A.) for Chemico-Biological Interactions, associate editor, Reviews in Biochemical Toxicology.

Dr. J. B. Pritchard: Trustee, Secretary and Member of Scientific Advisory and Nominating Committees, Mount Desert Island Biological Laboratory, Salsbury Cove, Maine; Visiting Scientist, C. V. Whitney Marine Laboratory, University of Florida, St. Augustine; Adjunct Associate Professor, Dept. of Pharmacology, University of Florida, Gainesville; invited speaker ASPET symposium on "Aquatic Animals as Models in Biomedical Research."

Dr. M. O. James: Adjunct Assistant Professor, Dept. of Pharmacology, University of Florida, Gainesville.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80001-07 LP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Microsomal Mixed-Function Oxidase Systems: Specificity and Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard M. Philpot	Research Chemist	LP NIEHS
Other:	Cosette S. Shull	Chemist	LP NIEHS
	Charles R. Wolf	Visiting Associate	LP NIEHS
	Jane E. Croft	Visiting Fellow	LP NIEHS

COOPERATING UNITS (if any)

Chemistry Branch

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Toxication-Detoxication

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	3	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The objective of this research project is to assess the factors responsible for differences in the substrate specificities among cytochrome P-450-dependent microsomal mixed-function oxidase systems (MFO) from various sources. Present work involves the purification of cytochrome P-450, NADPH-cytochrome c reductase, and lipid fractions from rabbit pulmonary and hepatic microsomes. The cytochrome P-450s are being examined by UV-visible spectroscopy, electron paramagnetic resonance spectroscopy, SDS-gel electrophoresis, and by activities in reconstituted systems. Structural and immunological properties of the cytochromes are also being investigated. The long-range objective of this work is to determine the influence of: 1) multiple forms of the enzymic components of the MFO system; 2) endogenous compounds; and 3) exogenous compounds (inhibitors and inducers) on the substrate specificities of MFO systems from different tissues and species.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Cytochrome P-450 is purified from rabbit pulmonary and hepatic microsomes by procedures developed in this laboratory. The purification of other enzymes and the preparation of phospholipids is done according to existing methods from the literature.

MAJOR FINDINGS AND PROPOSED COURSE: 1) The Rabbit Pulmonary Mixed-Function Oxidase System: A number of reactions carried out by rabbit pulmonary microsomal fractions appear to be a function of a single form of cytochrome P-450. This cytochrome, P-450_I, has been purified and used to elicit antibodies in goats. The antibodies, in the form of purified IgG fractions, inhibit all of the benzphetamine, ethylmorphine, and aminopyrine N-demethylation and 7-ethoxycoumarin O-deethylation activities in pulmonary microsomes. The second form of pulmonary cytochrome P-450, P-450_{II}, does not cross-react with the antibodies to P-450_I and the inhibited reactions can be assigned to P-450_I. Cytochrome P-450_I also catalyzes the hydroxylation of aniline and coumarin but is responsible for only about 50% of these reactions. Cytochromes P-450_I and P-450_{II} are different enzymes based on their apparent monomeric molecular weights, spectra, substrate specificity, immunological properties and structures.

2). The Rabbit Hepatic Mixed-Function Oxidase System. The form of rabbit hepatic cytochrome P-450 that is induced by phenobarbital, P-450_{PB}, is immunochemically identical to rabbit pulmonary cytochrome P-450_I. The hepatic cytochrome has been purified by the method developed for the purification of pulmonary cytochrome P-450_I. Final preparations contain 17-20 nmol P-450 per mg protein with yields of up to 20% of the total microsomal cytochrome. P-450_{PB} catalyzes the same reactions as does P-450_I and is responsible for nearly all of these activities in hepatic microsomes prepared from rabbits treated with phenobarbital. These activities are catalyzed in microsomes from untreated rabbits by at least two forms of cytochrome P-450, one of which is P-450_{PB}.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A full understanding of mixed-function oxidase systems is required if drug interaction, drug metabolism and disposition of ingested or inhaled environmental pollutants, and pesticide detoxification are to be fully comprehended.

PUBLICATIONS

Wolf, C. R., Szutowski, M., Ball, L., and Philpot, R. M.: The rabbit pulmonary monooxygenase system: Characteristics and activities of two forms of pulmonary cytochrome P-450. Chem.-Biol. Interactions 21: 29-43, 1978.

Patel, J. M., Wolf, C. R., and Philpot, R. M.: The interaction of 4-methylbenzaldehyde with rabbit pulmonary cytochrome P-450 in the intact animal, microsomes, and purified systems--destructive and protective reactions. Biochem. Pharmacol. In press.

Wolf, C. R., Smith, B. R., Ball, L., Serabjit-Singh, C. J., Bend, J. R., and Philpot, R. M.: The rabbit pulmonary monooxygenase system: Catalytic differences between two forms of cytochrome P-450 in the metabolism of benzo(a)pyrene. J. Biol. Chem. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80002-09 LP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Enzymes Metabolizing Chemicals: Chemical and Physiological Effectors of These Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Theodora R. Devereux	Research Biologist	LP NIEHS
	James R. Fouts	Chief	LP NIEHS
Other:	Reen Wu	IPA	LPFT NIEHS
	Richard M. Philpot	Research Chemist	LP NIEHS

COOPERATING UNITS (if any)

Laboratory of Pulmonary Function and Toxicology

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Toxication-Detoxication

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

It is the long-range purpose of this project to study how various chemicals and physiological changes affect xenobiotic metabolism by the body. This laboratory has concentrated its effort on the lung as a target organ for exposure to environmental stresses. Present studies include isolation of rabbit lung cell types for the purpose of studying localization of xenobiotic metabolism within the lung and toxication-detoxication mechanisms in individual cell populations. The following model enzyme systems are being used for study of individual xenobiotic metabolic pathways in lung cell populations: coumarin hydroxylase, 7-ethoxycoumarin deethylase, benzo(a)pyrene hydroxylase, and N,N-dimethylaniline N-oxidase. Different lung cell fractions (mixed cell populations) appear to have different metabolic profiles indicating possible differences in cytochrome content in the cell types. Cell fractions containing alveolar type II cells and nonciliated bronchiolar epithelial cells (Clara cells) are being further purified for detailed studies of mixed-function oxidase activity.

PROJECT DESCRIPTION

METHODS EMPLOYED: Various enzyme preparations, including trypsin and pronase, have been used for dispersal of rabbit lung cells. Populations of cells are separated from the cell digest according to their rates of sedimentation by the technique of centrifugal elutriation. Other methods employed for cell separation include density gradients, phase separation, differential attachment to tissue culture plates, and affinity chromatography. Cells are studied using light microscopic (including fluorescence microscopy) and electron microscopic techniques. Spectrophotometric, fluorometric, and radiometric methods are used to study cytochrome P-450 and to quantify metabolites of substrates added to cell suspensions.

MAJOR FINDINGS AND PROPOSED COURSE: Techniques are being developed to disperse and separate the many lung cell types in order to localize and study drug metabolism in individual cell populations. Present research is being directed toward obtaining relatively pure populations of alveolar type II cells and Clara cells since these cell types contain a majority of the pulmonary endoplasmic reticulum (where mixed-function oxidase activity seems to occur). Protease I (Sigma) is currently being used to obtain a cell digest consisting of 20-30% alveolar type II cells and about 5% Clara cells. The cells are separated by size in an elutriator centrifuge yielding a fraction which is 50-70% type II cells (< 2% Clara cells) and another fraction which is 25-30% Clara cells (about 10% type II cells). Recently, progress has been made in the identification of Clara cells under the light microscope using a nitroblue tetrazolium staining technique. The alveolar type II cell fraction has been found to be enriched in 7-ethoxycoumarin deethylase activity but almost lacking in coumarin hydroxylase activity. However, both these activities were enriched in the Clara cell fraction. Efforts are now being made to prove whether or not different cytochromes exist in these separate lung cell populations. Immunological studies with cell fractions and antibodies to the purified cytochromes are being used to localize the cytochromes. Separation and purification of the cell types are also being improved with new density gradients and methods which separate cells by surface charge. Xenobiotic metabolism is being measured in primary cultures of these cell fractions over time. Techniques to prevent mixed-function oxidase activity loss during culture are being studied. Also, attempts are being made to get pure cultures of alveolar type II cells and Clara cells started. Future research will focus on the study of toxication and detoxication mechanisms within isolated cell populations to understand the ways in which chemical and physicological stresses alter these systems in lung.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This research seeks to understand some of the factors that affect xenobiotic metabolism in tissues in contact with the environment. Use of lung cells to study drug metabolism may lead to a better understanding of lung damage and repair mechanisms. Comparisons will be made of cellular versus microsomal, purified, and isolated perfused lung xenobiotic metabolism systems to see where differences exist and what this may contribute to understanding toxication and detoxication mechanisms in the body. Studies of changes caused by steroids, metal ions, or other chemicals, or physiological changes (age) on these cellular systems may aid our understanding of when the body is more susceptible to toxic agents and, on a cellular basis, how the body handles these insults. Studies of xenobiotic metabolism in lung cell populations may give us a better understanding of the balance between toxication and detoxication mechanisms and the varied ways chemicals and physiological stresses can alter these systems and this balance.

PUBLICATIONS

Devereux, T. R. and Fouts, J. R.: The effect of dexamethasone treatment on N,N-dimethylaniline demethylation and N-oxidation in pulmonary microsomes from pregnant and fetal rabbits. Biochem. Pharmacol. 27: 1007-1008, 1978.

Devereux, T. R., Hook, G. E. R., and Fouts, J. R.: Foreign compound metabolism by isolated cells from rabbit lung. Drug Metab. Disp. 7: 70-75, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80003-06 LP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Xenobiotic-Metabolizing Enzyme Activity in Skin and Its Response to Environmental Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Roberta J. Pohl	Research Biologist	LP NIEHS
	James R. Fouts	Chief	LP NIEHS

COOPERATING UNITS (if any)

Biometry Branch

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Toxication-Detoxication

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XXX☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The project is designed to elucidate the role of xenobiotic-metabolizing enzymes in skin as mediators of toxicity of environmental agents. Mixed-function oxidase (including aryl hydrocarbon hydroxylase), glutathione S-transferase and UDP-glucuronyltransferase activities are measured in pieces of whole skin or epidermis, or in microsomes or cytosol from skin of hairless mice (Hrs/J) after exposure of the mice to ultraviolet radiation, polycyclic hydrocarbons, chlorinated hydrocarbons, etc. The wavelength of maximum absorbance in the carbon monoxide spectra of reduced cytochrome P-450 is identified after separating the cytochrome from contaminating mitochondrial pigments.

PROJECT DESCRIPTION

METHODS EMPLOYED: A procedure was developed to increase the yield of microsomal fraction from skin homogenate, from 2 to >4 mg protein per g skin, using a homogenizing medium of 125 mM KCl containing 20 mM potassium phosphate buffer, pH 7.7, and 0.2% bovine serum albumin. This preparation was unsuitable for assay of cytochrome P-450 because of contamination by cytochrome oxidase. A microsomal preparation which had no absorbance attributable to cytochrome oxidase in the carbon monoxide difference spectrum of reduced microsomes was obtained from epidermal cells isolated from skin digested with trypsin overnight in the cold. The cells were homogenized in a Potter-type homogenizer, sonicated 10 sec and isolated by centrifugation after aggregation by calcium and magnesium salts in 0.25 M sucrose.

The assay of 7-ethoxyresorufin deethylase activity was improved by adding bovine serum albumin to the incubation mixture and by using methanol to stop the reaction and precipitate the protein without binding of the metabolite.

MAJOR FINDINGS AND PROPOSED COURSE: Forty-eight hours after topical application of β-naphthoflavone (2.5 mg/mouse in 0.1 ml DMSO) to hairless mice, Hrs/J (Jackson Labs), 7-ethoxycoumarin deethylase (7-EC) was increased nearly twofold in microsomes from liver and skin. Interperitoneal treatment of mice with β-naphthoflavone in corn oil also modified the activity of 7-EC in microsomes from liver or skin. Inhibition or induction of activity was observed, depending on dose and time after administration.

Assay of microsomes from skin for aminopyrine demethylase activity, using a sensitive radiometric method, failed to detect any metabolism of aminopyrine.

The method (described in the previous subheading) for isolating microsomes from epidermal cells will be exploited for measuring cytochrome P-450 content in skin after exposure to environmental agents such as chlorinated hydrocarbons, ultraviolet radiation, organic solvents, and steroids. The effect of these agents on xenobiotic metabolism will also be further studied.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Persistent environmental pollutants, specifically chlorinated organic compounds, are often accumulated in skin. Many other biologically active chemicals are applied to the skin in medicaments, cosmetics, cleaning compounds, etc. Xenobiotic-metabolizing enzymes in the skin may have a role in the locally or systemically expressed toxicity of these compounds. Increased understanding of that role may lead to development of better systems to assess toxicity of chemicals. Manipulation of xenobiotic metabolism may be used to maximize the beneficial effects of chemicals applied to the skin while minimizing toxic reactions.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80005-06 LP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

In Vitro Metabolism of Xenobiotics by Selected Marine Species

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John R. Bend	Visiting Scientist	LP NIEHS
	Margaret O. James	Senior Staff Fellow	LP NIEHS
Other:	Louise M. Ball	Visiting Fellow	LP NIEHS
	Gary L. Foureman	Biologist	LP NIEHS
	Roberta J. Pohl	Research Biologist	LP NIEHS
	Richard M. Philpot	Research Chemist	LP NIEHS
	Barbara J. Stetson	Biological Lab Tech	LP NIEHS
	Peter J. Little	Visiting Fellow	LP NIEHS

COOPERATING UNITS (if any)

Biometry Branch; Environmental Chemistry Branch; C. V. Whitney Marine Laboratory, University of Florida.

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Marine Pharmacology and Biomedicine

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.9	1.8	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

XX

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

We are investigating the biotransformation of foreign organic compounds in hepatic and extrahepatic tissues of vertebrate and invertebrate marine species from coastal Maine and Florida. Both cytochrome P-450-dependent microsomal mixed-function oxidases and alkene and arene oxide-metabolizing enzymes (epoxide hydrase and glutathione S-transferases) are being characterized in control fish and in fish pre-exposed to environmental contaminants such as polycyclic aromatic hydrocarbons, dioxins or polyhalogenated biphenyls.

PROJECT DESCRIPTION

OBJECTIVES: 1) To study possible reasons for the variations in hepatic xenobiotic oxidative metabolism, cytochrome P-450 content, and epoxide-metabolizing enzymes observed in marine fish, especially sheepshead (*Archosargus probatocephalus*).

2) To monitor individual teleost and elasmobranch fish for exposure to 3-methylcholanthrene (3-MC)-like inducing agents, by assaying hepatic benzo(a)-pyrene hydroxylase activity in the presence and absence of the *in vitro* probe 7,8-benzoflavone, and attempt to correlate induction with possible exposure to environmental inducing agents, and to compare these effects in Maine vs. Florida.

3) To study how different classes of chemicals, which act as inducers of hepatic xenobiotic metabolism in mammals, affect hepatic xenobiotic metabolism in marine species.

4) To further investigate xenobiotic-metabolizing pathways in the spiny lobster (*Panulirus argus*) and lobster (*Homarus americanus*), especially cytochrome P-450-dependent oxidation.

5) To measure taurine N-acyltransferase activity in several marine species and to characterize the enzyme in a suitable aquatic species.

6) To characterize the hepatic mixed-function oxidase (MFO) system and glutathione *S*-transferases of representative species from Maine and Florida, before and after pretreatment with polycyclic hydrocarbon-like inducing agents.

7) To study mercapturic acid biosynthesis in representative aquatic species.

METHODS EMPLOYED: 1) Following up previous results which suggested that cytochrome P-450 (P-450) content of hepatic microsomes was somewhat lower in female sheepshead than in males during the spawning season, we grouped fish according to gonadosomatic index and found lower P-450 content in spawning females than in nonspawning females or males. However, earlier results indicating lower epoxide hydrase activity in females during the spawning season were not confirmed when more sheepshead were studied. Individual variation was high for epoxide hydrase activity in all groups of fish.

We propose to collect more data on sheepshead during the fertile season in order to establish whether or not the observed trends are real. Treatment of fish with hormones to induce gonadal maturation may then be used to study this phenomenon.

2) A substantial number (67/166) of untreated sheepshead assayed between January 1978 and April 1979 were found to have elevated benzo(a)pyrene hydroxylase (AHH) activities which were inhibited *in vitro* by 7,8-benzoflavone,

indicating exposure to a 3-MC-like inducing agent. Eighteen of the induced fish were freshly caught and held less than 4 weeks before sacrifice, suggesting environmental exposure to inducing agent(s). The remaining induced fish were held for longer periods at Whitney Marine Lab. Forty of the induced fish were held in a tank which sustained rapid growth of algae (predominantly *Ulva lettuca*). Algae were found in the sheepshead's intestines at sacrifice. It seemed possible that a component of algae was responsible for the observed induction of AHH and the elevation of P-450 content found in those sheepshead whose intestines contained algae.

Future plans include collaborating with the Environmental Chemistry Branch at NIEHS to determine what agents could be causing the observed induction of AHH and sometimes P-450 in untreated fish.

3) We previously found induction of hepatic MFO activity in some Maine winter flounder (*Pseudopleuronectes americanus*), but only a few fish were tested. In the present study we determined 7-ethoxyresorufin (7-ERF) and AHH (in the absence and presence of 10^{-4} and 5 x 10^{-4} M 7,8-benzoflavone (BF) activities in liver homogenate from 172 flounder between June and August, 1978. Large variations (70 to 80-fold for AHH and in excess of 60-fold for 7-ERF, respectively) were observed in hepatic MFO activities of these fish. The higher the AHH activity, the greater was the inhibition by *in vitro* BF, and the lower the AHH activity, the greater the stimulation by BF. There was no major difference between male and female flounder with any activity assayed. A positive correlation (0.83) existed between hepatic AHH and 7-ERF activities in individual flounder. The causative factor for the polycyclic hydrocarbon-type induction observed in 50% of the wild flounder sampled has not yet been elucidated, although it could be exposure to certain pollutants. Since it occurred in all groups tested between June and August, it does not appear to be related to spawning.

We propose to assay liver homogenates of these flounder with highly induced AHH and 7-ERF activities for polychlorinated biphenyls (PCB) and polycyclic aromatic hydrocarbon residue levels, which will be compared with pollutant content of livers from fish showing no induction. We will also use SDS-polyacrylamide gel electrophoresis to check livers of induced fish for a novel form of cytochrome P-450, with catalytic properties similar to skate and rat cytochrome P-448.

4) Further study of 3-MC induction in sheepshead showed that a dose of 10 mg/kg induced AHH activity in sheepshead as early as 24 hr after i.p. injection, even at water temperatures of 14°C. The P-450 content of hepatic microsomes was usually increased in fish induced by i.p. injection of 3-MC, but the increase was rarely statistically significant for any one group of sheepshead. However, i.m. injection of 3-MC into sheepshead caused a statistically significant 2.5-fold increase in mean P-450 content of treated fish over controls, and a shift to the red of 1 to 2 nm in the spectral maximum of the CO-reduced difference spectrum. AHH activity was not increased any more in the i.m.-treated fish than in the i.p.-treated fish.

Administration of phenobarbital to sheepshead at doses of 100 mg/kg narcotized the fish for 2 to 4 hr after dosing, but three such doses had no effect on P-450 content, AHH activity, benzphetamine N-demethylase activity or 7-ethoxycoumarin O-deethylase activity in hepatic microsomes. Similarly, the PCB and polybrominated biphenyls (PBB) isomers which cause type 1 (phenobarbital-like) induction in mammals, i.e., 2,2',4,4',5,5'-hexahalogenated biphenyls, also had little effect on hepatic xenobiotic-metabolizing enzymes in sheepshead hepatic microsomes.

We propose to determine how long hepatic AHH activity remains induced in sheepshead treated with a single dose of 3-MC. Conditions established by these studies for maximum induction of P-450 and AHH in hepatic microsomes will be used in experiments where *in vivo* metabolism of foreign chemicals,, e.g., benzo(a)pyrene, is compared in induced and noninduced fish, and also in purification of P-450 from induced sheepshead. Other more potent chemicals reported to induce in the same way as phenobarbital will be studied in sheepshead and little skate (*Raja erinacea*), when they become available.

5) The major form of hepatic cytochrome P-450 isolated from the male little skate (representing 80-90% of the total liver cytochrome content) was reconstituted as an active MFO system with a standard preparation of rabbit liver NADPH-cytochrome *c* reductase, sodium cholate and rabbit phospholipid or synthetic dilauroylphosphatidylcholine. This system was able to metabolize benzo(a)pyrene at a rate of 0.1 nmol/min/nmol cytochrome (assayed by fluorescence), with apparent K_m approximately 10 μM. Benzo(a)pyrene metabolism was stimulated threefold by addition of 10^{-4} M BF to the incubation medium. This compares well with values of 40 pmol/min/mg protein, K_m = 6 μM, and threefold stimulation with 10^{-4} M BF, which were obtained previously with fresh male skate microsomes. Of the two pools of cytochrome resolved from the livers of male dibenzanthracene (DBA)-treated little skates, the P-448 turned over benzo(a)pyrene at 0.8 nmol/min/nmol cytochrome in the reconstituted system and was inhibited 80% by 10^{-4} M BF as were the fresh microsomes from male DBA-treated little skates; the rate of benzo(a)pyrene metabolism with the P-451 was 80 pmol/min/nmol cytochrome. Identification of the benzo(a)-pyrene metabolites by HPLC indicated that with both untreated and induced skate liver microsomes the predominant metabolic products (50% of the total) were 3-hydroxybenzo(a)pyrene and quinones derived from this phenol, with the quinones being formed by the induced microsomes being somewhat more numerous. Metabolism at the 9,10 position accounted for about 20% of the total. Similar results were obtained with female skates. Thus, the induced and uninduced MFO systems in the little skate liver differ in the rate of metabolism rather than in the nature of the metabolites formed, and the cytochromes which have been isolated are able, in a reconstituted system, to account for these differences in rate.

It is proposed to further purify and characterize the hepatic cytochromes P-450 from male and female induced and untreated little skates, to investigate the interaction of uninduced cytochrome P-450 with BF and the stimulation or inhibition of benzo(a)pyrene metabolism by this compound.

6) The glutathione S-transferase activity of little skate liver cytosol has been separated into five enzymes (1 major and 4 minor), two of which (the major and 1 minor) are homogeneous as judged by SDS-polyacrylamide gel electrophoresis (PAGE). Separation was achieved by eluting the transferase activity (with 1,2-dinitro-4-chlorobenzene) from a DEAE-cellulose column with a KCl gradient. Further purification was achieved by (a) passing these peaks eluted from DEAE-cellulose columns individually through a glutathione-Epoxy-Sepharose column and (b) eluting the enzyme from this column with glutathione. All enzymes differ from one another in isoelectric point, 4 of the 5 having $pI < 7.0$. SDS-PAGE showed that the two purified enzymes differed from one another in monomolecular unit weight, the major enzyme being about 32,000 daltons and the minor being about 28,500 daltons. Four of the five enzymes differed markedly in substrate specificities with the major enzyme exhibiting a specific activity with benzo(a)pyrene 4,5-oxide of 16 μmol/min/mg protein.

The glutathione S-transferases from little skate liver will be compared to mammalian glutathione S-transferases using gel electrophoresis, isoelectric focusing, immunological, and amino acid analysis procedures as well as catalytic activity with a number of electrophilic substrates including alkene and arene oxides.

7) Cytochrome P-450 assayed in hepatic microsomes from little skate and New Zealand White rabbits was stable in both species after exposure for 5 min to temperatures as high as 45°C. NADPH-cytochrome c reductase activity in the microsomes from little skate was decreased after exposure to 35-40°C while activity in microsomes from rabbit was unchanged until 45°C was used. The decrease in NADPH-cytochrome c reductase activity after exposure to elevated temperatures correlated with the decrease in benzphetamine N-demethylase activity in microsomes from both species. NADPH-cytochrome c reductases isolated from hepatic microsomes of the two species were equally susceptible to inactivation by exposure to elevated temperatures. The molecular weights obtained by SDS-gel electrophoresis of the isolated enzymes were not the same, however, and the enzyme from little skate did not react with antibody to rabbit NADPH-cytochrome c reductase in an Ouchterlony gel.

Purified NADPH-cytochrome c reductases will be reconstituted with purified rabbit hepatic cytochrome P-450 in the presence of synthetic lipid, and the effect of exposure of the systems to elevated temperatures on benzphetamine N-demethylase activity will be determined. These experiments should help to elucidate the factor(s) responsible for the difference in thermolability of MFO between the two species.

8) We have previously shown that the low activities of cytochrome P-450-dependent mixed-function oxidations in hepatopancreas microsomes from the spiny lobster are not due to their lack of P-450 or to their ability to bind substrates to the cytochrome, but are probably due to the very low NADPH-cytochrome P-450 reductase activities of hepatopancreas microsomes.

Hepatopancreas microsomes were also found to contain an inhibitor of mixed-function oxidation, probably a protease. Further support for these conclusions was gained by solubilizing hepatopancreas microsomes and subjecting them to DEAE-cellulose chromatography in the presence of the protease inhibitor, PMSF. About 50% of the added P-450 was recovered from the column, in two peaks. When the column was run in the absence of PMSF, only 2% of the added P-450 was recovered. No NADPH-cytochrome c reductase activity was recovered from the column, even in the presence of PMSF. Both cytochrome P-450 fractions contained other proteins, including epoxide hydrase. The P-450 fractions, when reconstituted with rabbit liver cytochrome P-450 reductase and dilauroylphosphatidylcholine, supported oxidation of benzphetamine, benzo(a)pyrene and ethoxyresorufin.

In the future, we hope to further purify the spiny lobster P-450, using established procedures or modifications as necessary. We plan to study the metabolism of benzo(a)pyrene by the spiny lobster P-450 in reconstituted systems and compare the metabolites with those formed in vivo, if indeed benzo(a)pyrene is metabolized in vivo by the spiny lobster. We also plan to ascertain whether or not the spiny lobster P-450 can be induced by chemicals such as 3-MC.

9) Further studies of taurine N-acyltransferase activity in fish kidney showed that higher specific activities were present in the posterior than in the anterior kidney of teleost fish (we studied the mullet, Mugil cephalus, and the southern flounder, Paralichthys lethostigma). This correlated with the known physiology and anatomy of fish kidney, since the posterior kidney functions better as an excretory organ than the anterior kidney, which is largely hematopoietic. Taurine N-acyltransferase activity was routinely studied using phenylacetylCoA as substrate, but we have now shown that benzoyl-CoA can also combine with taurine in a reaction catalyzed by the same fraction of kidney mitochondria.

We plan to purify the taurine N-acyltransferase enzyme from fish kidney and compare this enzyme with glycine N-acyltransferase from the rat or rabbit.

10) Preliminary investigations have shown that the cytochrome P-450 and NADPH-cytochrome c reductase activity in solubilized hepatic microsomes from control sheepshead can be separated using DEAE-cellulose column chromatography.

We plan to further purify the P-450 from untreated and 3-MC treated sheepshead using appropriate modifications of published methods and to purify the cytochrome P-450 reductase using the 2',5'-ADP-agarose affinity column procedure. The purified components will be used in reconstitution experiments and also in comparative studies of these enzymes from different aquatic species.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The marine ecosystem is contaminanted with substantial amounts of pesticides, industrial by-products, and other synthetic organic chemicals which are produced in huge quantities by our technologically oriented society. The ability or inability of marine speces, particularly those that are edible or those which are important in the aquatic food chain, to biotransform and excrete these xenobiotics is relevant both to the subsequent fate of these species (in the face of increasing pollution) and to their potential value and hazard as direct and indirect foodstuffs for man. Comparison of the enzymes involved in metabolizing xenobiotics in different species should lead to a better mechanistic understanding of the overall effects of xenobiotics on the animal, and of animals on xenobiotics.

Moreover, a detailed understanding of metabolic activation and deactivation pathways in marine animals may allow us to predict those species most likely to be affected by carcinogens, mutagens, teratogens, or cytotoxins in the environment and allow us to use them as sentinel or early warning indicators of toxic environmental contaminants.

PUBLICATIONS

Bend, J. R. Foureman, G. L., and James, M. O.: Partially induced hepatic mixed-function oxidase systems in individual members of certain marine species from coastal Maine and Florida. Second International Symposium on Aquatic Pollutants, the Netherlands, September, 1977. London, Pergamon, pp. 483-486.

Bend, J. R. and James, M. O: Xenobiotic metabolism in freshwater and marine species. In Malins, D. C. and Sargent, J. R. (Eds.): Biochemical and Biophysical Perspectives in Marine Biology. Vol. 4. New York, Academic, 1978, pp. 125-188.

James, M. O., Khan, M. A. Q. and Bend, J. R.: Hepatic microsomal mixed-function oxidase activities in several marine species common to coastal Florida. Comp. Biochem. Physiol. 62C: 155-164, 1979.

James, M. O., Bowen, E. R., Dansette, P. M. and Bend, J. R.: Epoxide hydrase and glutathione S-transferase activities with selected alkene and arene oxides in several marine species. Chem-Biol. Interactions. In press.

Bend, J. R., Ball, L. M., Elmamlouk, T. H., James, M. O. and Philpot, R. M.: Microsomal mixed-function oxidation in untreated and polycyclic aromatic hydrocarbon-treated marine fish. Symposium on Metabolism and Disposition of Pesticides and Other Chemicals in Aquatic Organisms. 176th Meeting of the American Chemical Society, ACS Symposium Series, 1978. In press.

James, M. O. and Bend, J. R.: Effect of polynuclear aromatic hydrocarbons and polyhalogenated biphenyls on hepatic mixed-function oxidase activity in marine fish. Proceedings of the symposium titled Carcinogenic Polynuclear Aromatic Hydrocarbons in the Marine Environment, 1978. In press.

Bend, J. R., Foureman, G. L., Ben-Zvi, Z., Dostal, L., Koo, J. O., and Fouts, J. R.: Heterogeneity of hepatic benzo(a)pyrene hydroxylase (aryl hydrocarbon hydroxylase) and 7-ethoxyresorufin deethylase activities in individual winter flounder, _Pseudopleuronectes americanus_, from coastal Maine. _Bull. Mt. Desert Island Biol. Lab_. In press.

Bend, J. R., James, M. O., and Pritchard, J. B.: Aquatic Toxicology. In Guthrie, F. E. and Perry, J. J. (Eds.): _Environmental Toxicology_. New York, Elsevier/North-Holland. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 80006-08 LP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Environmental Contaminants: Uptake, Distribution, Metabolism, Excretion, and Storage Sites in Marine Species

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John R. Bend	Visiting Scientist	LP NIEHS
	Margaret O. James	Senior Staff Fellow	LP NIEHS
Other:	John B. Pritchard	Research Physiologist	LP NIEHS
	Gary R. Foureman	Biologist	LP NIEHS
	Peter J. Little	Visiting Fellow	LP NIEHS

COOPERATING UNITS (if any)
Biometry Branch

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Marine Pharmacology and Biomedicine

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The long-range objective of this project is to study the in vivo uptake, distribution, metabolism, and excretion of single, purified radiolabeled environmental contaminants, such as 2,4,5-trichlorophenoxyacetic acid, polychlorinated biphenyl isomers, and hydrocarbons, in vertebrate and invertebrate marine species that serve as human food sources. The role of environmental temperature and exposure to other pollutants on the processes involved are also investigated. Particular attention is focused upon potential carcinogens, mutagens, teratogens, and cytotoxins that may occur as food residues due to exposure of aquatic animals to environmental pollutants.

PROJECT DESCRIPTION

METHODS EMPLOYED: At various times after administration (generally parenteral) of radiolabeled compounds, specimens are sacrificed, plasma and tissues are removed, and aliquots are oxidized and/or solubilized prior to liquid scintillation counting. Urinary and biliary excretion rates are also monitored where appropriate. Metabolites are identified by standard procedures including HPLC. Spiny lobster (Panulirus argus) "urine" is obtained at the time of sacrifice by inserting a syringe into the bladder at the base of each green gland.

MAJOR FINDINGS AND PROPOSED COURSE: 1) ^{14}C-Benzoic acid was excreted slowly in urine by the southern flounder, Paralichythys lethostigma. Excretion appeared to be zero order, at least over 96 hr, by which time approximately 60% of the dose was excreted. More than 95% of the urinary ^{14}C was a polar metabolite, probably benzoyltaurine, but definitely not hippuric acid. ^{14}C-2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) were excreted in southern flounder urine with excretion $t_{1/2}$ between 24 and 48 hr. Both 2,4-D and 2,4,5-T were excreted partly as taurine conjugate and partly as free acid.

The proposed course is to confirm the identity of the benzoic acid metabolite since earlier studies by others suggested that benzoic acids were excreted in fish as glycine conjugates.

Other proposed studies include collaborating with Dr. J. B. Pritchard to study factors affecting excretion of xenobiotic carboxylic acids, including the effect of water temperature on excretion rates.

2) Comparison of the fates of ^{14}C-labeled 2,4-D, 2,4,5-T, DDA and phenylacetic acid in the spiny lobster showed that all acids were taken up from hemolymph into hepatopancreas and green gland. The relative amounts of each acid taken up by hepatopancreas or green gland determined the extent of metabolism and rate of excretion. 2,4-D and 2,4,5-T were taken up better by green gland than by hepatopancreas and were rapidly excreted, unmetabolized, in "urine." The portion of the 2,4-D or 2,4,5-T dose taken up by hepatopancreas was metabolized to a more polar conjugate and excreted more slowly than the portion taken up by green gland. More of the dose of phenylacetic acid was taken up by hepatopancreas than by green gland, and the phenylacetic acid metabolite was excreted very slowly from hepatopancreas. The portion of phenylacetic acid which was excreted in 6-hr urine was more than 90% unmetabolized free acid. DDA had excretion properties between 2,4-D and phenylacetic acid.

We plan to identify the metabolite of phenylacetic acid in hepatopancreas and determine if the metabolites of 2,4-D, 2,4,5-T and DDA are similar. We also wish to obtain a more detailed pharmacokinetic analysis of the excretion of these xenobiotics, for purposes of comparison with other species, including man.

3) Further studies of the disposition of ^{14}C-hexadecane in the spiny lobster revealed that the shell took up considerable amounts of the dose (18% at 24 hr after dosing) and that the shell ^{14}C was depurated slowly (13% in shell at 1 week after dose). It appeared that the ^{14}C stored in hepatopancreas was slowly metabolized.

We plan to identify the metabolites of hexadecane and determine if the hexadecane stored in shell is metabolized.

We also plan to study the fate and metabolism of other chemicals, especially benzo(a)pyrene, in the spiny lobster, for comparison to complete work in the lobster (Homarus americanus).

4) ^{14}C-Benzo(a)pyrene was taken up mainly into the hepatopancreas (HP) of the Maine lobster following pericardial injection and eliminated very slowly with 64% of the dosed radioactivity being present (mostly in HP) after 6.5 weeks. Time-dependent elimination from all tissues was similarly slow, e.g., 3.9% of the radioactive dose was present in tail muscle after 6.5 weeks. An exception to this depuration was noted in female gonads where the radioactivity present increased with time; 2.3% of the dose was present at 6.5 weeks as compared with 0.9% and 0.6% at the earlier 1- and 2-week time points, respectively.

HPLC analyses showed the parent compound, benzo(a)pyrene, to be the major radioactive component in hexane extracts of homogenized HP at 3 weeks (95%) and 6.5 weeks (83%) after treatment. Radioactive phenolic metabolites were also present in this organic phase and increased from 3% at 3 weeks to 17% at 6.5 weeks. Identical analytical procedures applied to tail muscle homogenate showed benzo(a)pyrene was also the major component in the hexane extracts and that more phenolic metabolites were present than in HP: 39% at 2 days and 50% at 6.5% weeks, respectively.

Studies will continue on the uptake, metabolism, and elimination of selected radiolabeled aliphatic and aromatic constituents of crude oil, using at least one vertebrate and invertebrate species. The effect of pre-exposure of experimental animals to foreign organic chemicals, such as polychlorinated biphenyls, chlorinated insecticides and hydrocarbons, and to heavy metals on the subsequent uptake, metabolism and elimination of these aliphatic and aromatic radiolabeled crude oil constituents will also be studied in Maine and Florida marine animals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Marine pollution by chemicals such as crude and refined petroleum products, pesticides, polychlorinated biphenyls, polybrominated biphenyls, and polycyclic aromatic hydrocarbons is a problem of significant importance to human health since many aquatic organisms concentrate such pollutants and man relies upon these species for substantial quantities of food. Carefully controlled pharmacokinetic experiments will identify species that can be sampled in the field to monitor both short- and long-term pollutant levels. The study of interaction between environmental contaminants and other xenobiotics, including drugs, food additives, and pesticides, is important since mixtures of such pollutants are always encountered in the environment. Such investigations in marine species, with regard to competition for metabolic and excretion pathways or storage sites, may assist in understanding increased (or decreased) toxicity of pollutant-drug mixtures as they affect higher animals, including man.

PUBLICATIONS

James, M. O.: Taurine conjugation of carboxylic acids in some marine species. In Aitio, A. (Ed.): Conjugation Reactions in Drug Biotransformation. Amsterdam, Elsevier/North-Holland, 1978, pp. 121-129.

Pritchard, J. B. and James, M. O.: Determinants of the renal handling of 2,4-dichlorophenoxyacetic acid (2,4-D) by winter flounder. J. Pharmac. Exp. Ther. 208: 280-286, 1979.

Foureman, G. L., Ben-Zvi, Z., Dostal, L., Fouts, J. R. and Bend, J. R.: Distribution of ^{14}C-benzo(a)pyrene in the lobster, *Homarus americanus*, at various times after a single injection into the pericardial sinus. Bull. Mt. Desert Island Biol. Lab. In press.

Lech, J. J. and Bend, J. R.: The relationship between metabolism of xenobiotic chemicals and toxicity in aquatic species. Symposium on Aquatic Toxicology. 18th Annual Meeting of the Society of Toxicology. Environ. Hlth. Persp. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80007-08 LP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Hepatic and Extrahepatic Conjugation and Oxidation Metabolic Pathways for Xenobiotics in Mammals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John R. Bend	Visiting Scientist	LP NIEHS
	Brian R. Smith	Senior Staff Fellow	LP NIEHS
Other:	Hasan Mukhtar	Visiting Associate	LP NIEHS
	Oscar Hernandez	Visiting Associate	ECB NIEHS
	Louise M. Ball	Visiting Fellow	LP NIEHS
	John L. Plummer	Visiting Fellow	LP NIEHS
	Boris Yagen	Visiting Scientist	LP NIEHS
	Gary L. Foureman	Biologist	LP NIEHS
	Richard M. Philpot	Research Chemist	LP NIEHS
	Richard Cox	Chemist	CB NIEHS

COOPERATING UNITS (if any)

Biometry Branch; Environmental Chemistry Branch

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Toxication-Detoxication

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.6	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Several arene and alkene oxides are known to react covalently with macromolecules, including nucleic acids, and to transform cells in vitro, suggesting their role as ultimate carcinogens, mutagens, and cytotoxins. We are studying the mixed-function oxidases, which convert unsaturated hydrocarbons to epoxides, and the further metabolism of arene and alkene oxides by soluble fraction glutathione S-transferases and microsomal epoxide hydrase in hepatic and extrahepatic tissues. The relative quantitative importance of the two pathways for epoxide metabolism is being studied in vitro in intact pulmonary cells, and in isolated perfused rat liver and rabbit lung preparations.

PROJECT DESCRIPTION

OBJECTIVES: To characterize in intact tissue the enzymatic pathways responsible for the metabolic conversion of polynuclear aromatic hydrocarbons to epoxides and for the decomposition of these reactive intermediates; 2) to assess the importance of these enzyme systems in protecting tissues from reactive intermediates; 3) to evaluate the influence of cellular integrity and intracellular location of enzymes on the metabolism of reactive intermediates; 4) to correlate dosage levels with activity of epoxide-metabolizing pathways and toxicity (including histopathology and covalent binding of radioactivity to macromolecules) and rates of excretion in isolated perfused organs; 5) to study developmental patterns of epoxide metabolism in germinal and steroidogenic tissues; and 6) to investigate position-specific hydroxylation of model substrates in reconstituted mixed-function oxidase (MFO) and other *in vitro* systems.

METHODS EMPLOYED: Differential centrifugation; spectroscopic, fluorometric, and radiochemical assays; organ perfusion procedures; ion exchange; thin-layer, paper, and high-pressure liquid chromatography; and synthetic organic chemistry.

MAJOR FINDINGS AND PROPOSED COURSE: Major findings -- 1) The ability of rabbit lung to metabolize benzo(a)pyrene (BP) at the microsomal level was studied using HPLC and liquid scintillation counting to identify and quantitate metabolites. Total rates of metabolism were of the order of 0.1 nmol product/min/mg microsomal protein, with 3-hydroxy BP being the major product (55-60% of the total metabolites). This is consistent with rates and profiles seen earlier in isolated perfused lungs and also with reconstituted purified rabbit pulmonary cytochrome P-450. Treatment of the rabbits with inducing agents (3-methylcholanthrene or sodium phenobarbital) did not significantly alter the overall rates of BP metabolism, but caused a marked increase (3-methylcholanthrene) or decrease (phenobarbital) in the proportion of metabolism occurring at the 9,10 position of BP. The formation of individual metabolites could be affected by addition of specific inhibitors to the incubation medium: 7,8-benzoflavone (α-naphthoflavone) almost totally abolished production of the *trans*-9,10-dihydroxy-9,10-dihydrobenzo(a)pyrene (9,10-dihydrodiol), and 2-diethylaminoethyl-2,2-diphenylvalerate HCl (SKF 525-A) did likewise for *trans*-4,5-hydroxy-4,5-dihydrobenzo(a)pyrene (4,5-dihydrodiol).

2) Treatment of rabbits with *p*-xylene (1 g/kg, i.p.) or *p*-methylbenzaldehyde (50 mg/kg in marginal ear vein) did not affect *in vitro* pulmonary epoxide hydrase activity but reduced BP metabolism concurrently with destruction of lung cytochrome P-450.

3) Previous investigations in our laboratory have shown glutathione (GSH) conjugates to be the major metabolites produced by perfused rat livers administered styrene oxide (10, 50, 100 or 500 μmol), indicating that the GSH *S*-transferases are a very important enzymatic pathway for styrene oxide metabolism Styrene oxide biotransformation was further investigated in perfused livers

depleted of GSH in order to delineate the importance of epoxide hydrase protection against styrene oxide-induced hepatotoxicity. GSH depletion was accomplished by treating rats with diethyl maleate 25 min prior to surgical removal of the livers, which consumed greater than 90% hepatic GSH. GSH was not significantly regenerated over 60 min of liver perfusion with or without styrene oxide. GSH-depleted livers administered styrene oxide (50, 100, or 500 μmol) produced substantially smaller amounts of thioether conjugates than did similarly treated livers from control animals. Styrene glycol was produced at an accelerated rate and for a greater duration of time by GSH-depleted livers than by untreated livers. In addition, greater quantities of styrene glycol-β-glucuronide were produced by GSH-depleted livers. Covalent binding of styrene oxide-derived radioactivity to DNA, RNA, and protein was no more extensive in GSH-depleted livers than in control livers given identical amounts of styrene oxide.

These results demonstrate that epoxide hydrase can compensate for diminished GSH S-transferase activity in GSH-depleted livers and can effectively protect the perfused rat liver from acute styrene oxide-induced toxicity.

4) BP 4,5-oxide (5 μmol) was rapidly biotransformed by perfused rat livers, and the products (BP 4,5-dihydrodiol and BP 4,5-oxide:thioether conjugates) were excreted in the bile and/or released into the circulation. The perfused rat livers could remove BP 4,5-dihydrodiol, but not BP 4,5-oxide:glutathione conjugates, from the circulation. BP 4,5-dihydrodiol was conjugated with β-glucuronic acid, but not sulfuric acid, and subsequently excreted in the bile. The maximum biliary excretion rate for BP 4,5-oxide metabolites was about 100 nmol/min.

BP 4,5-oxide-derived radioactivity was covalently bound to tissue DNA, RNA, and protein. Covalent binding to protein (14.3 ± 0.8 pmol/mg dry wt; x ± S.D., N = 3) was more extensive than binding to RNA or DNA (1.93 ± 0.50 or 0.44 ± 0.10, pmol/mg dry wt, respectively; x ± S.D., N = 3). Histological examination of livers administered BP 4,5-oxide (5 μmol) revealed extensive vacuolation (and nuclear contraction) in periportal hepatocytes. This damage may be associated with the covalent interaction of BP 4,5-oxide with cellular macromolecules.

5) A method has been developed for the characterization of small quantities of thioether conjugates (GSH, cysteinylglycine, cysteine, and N-acetylcysteine conjugates). The metabolite is treated with acid or alkali and the liberated amino acids are converted to their phenylthiohydantoin derivatives which are identified by HPLC.

This technique was used in an investigation of the biliary metabolites of BP 4,5-oxide in rats. After injection of BP 4,5-oxide (5 μmol) into the portal veins of four rats, 64.2 ± 9.0% (mean ± S.D.) of the administered radioactivity appeared in the bile within 40 min. The major metabolite was a GSH conjugate, which accounted for 57.4 ± 15.7% of the excreted radioactivity. A mercapturic acid made up a further 7.7 ± 9.6%, and 15.1 ± 0.9% consisted of the β-glucuronide of BP 4,5-dihydrodiol. The BP 4,5-dihydrodiol:glucuronide was identified by cochromatography (HPLC) with a biosynthetic standard and by the detection of BP 4,5-dihydrodiol after (but not before) treatment of the bile with β-glucuronidase. No metabolites were liberated by treatment of the bile with aryl sulfatase (Abalone) in the presence of saccharolactone.

6) An HPLC system for the identification and quantitation of water-soluble metabolites of BP 4,5-oxide has been developed using characterized thioether conjugates as standards. The metabolite profile in bile may now be rapidly determined, allowing detailed investigation of the factors affecting its pathways of detoxication.

This method has also proven useful in the identification and quantitation of thioether conjugates produced from BP by the isolated perfused rabbit lung.

7) BP 4,5-oxide was conjugated with glutathione and hydrated by perfused rabbit lungs at rates of 23.8 ± 2.3 and 7.60 ± 1.14 nmol/min/g of tissue, respectively. Rabbit lung also converted the BP 4,5-oxide:GSH S-transferase conjugate to the mercapturic acid (N-acetylcysteine) derivative. The metabolites were characterized by HPLC. Perfused rabbit lungs apparently lacked the ability to conjugate the BP 4,5-oxide hydration product, BP 4,5-dihydrodiol, with sulfuric or glucuronic acid. The BP 4,5-dihydrodiol slowly diffused into the perfusion medium and showed no tendency to accumulate in the tissue. Covalent binding of BP 4,5-oxide-derived radioactivity to tissue DNA and RNA (3.59 ± 0.81 and 29.6 ± 14.7 pmol/mg dry wt, respectively; x ± S.D., N = 3) was more extensive than that reported for similarly treated perfused rat livers. The overall BP 4,5-oxide biotransformation rate was about three times the estimated rate of arene oxide formation from BP by perfused rabbit lungs. This finding suggests that factors in addition to the balance between toxication and detoxication pathways in pulmonary tissue may contribute to the susceptibility of pulmonary tissue to polycyclic aromatic hydrocarbon-induced toxicity.

8) Kinetic parameters of rabbit pulmonary epoxide-metabolizing enzymes for BP 4,5-oxide as substrate were determined using lung subcellular fractions: GSH transferases (cytosolic fractions), Km = 1.72 ± 0.15 μM and Vmax = 2.84 ± 1.02 nmol/min/mg of protein; epoxide hydrase (microsomal), Km = 2.08 ± 0.30 μM and Vmax = 0.789 ± 0.192. Soluble and microsomal protein yields were 37.0 ± 3.9 and 18.4 mg/g of tissue, respectively (corrected for microsomal protein loss by determining cytochrome P-450 recovery). Knowing these yields allowed calculation of whole kinetic paramters: GSH S-transferase, Km = 63.6 nmol/g, Vmax = 105.1 nmol/min/g; epoxide hydrase, Km = 38.3 nmol/min/g, Vmax = 14.5 nmol/min/g. The calculated intrinsic extraction ratio of rabbit lung for BP 4,5-oxide was 0.090 ± 0.009. The observed intrinsic extraction ratio of rabbit lung for BP 4,5-oxide was 0.093 ± 0.031. These results indicate that enzymatic parameters determined by in vitro methods can be used to estimate whole organ metabolic activities if the enzyme content in the tissue is accurately known.

9) Impomeanol, a lung edema-inducing factor occurring in moldy sweet potatoes, was rapidly transformed by perfused rabbit lungs to a reactive metabolite(s) that became covalently bound to tissue macromolecules (DNA, RNA, and protein) or was conjugated with GSH. Covalent binding to RNA and protein was extensive (1.5 and 2.6 nmol/mg dry wt., respectively) at a concentration of 55 μM ipomeanol in the perfusate. Covalent binding to RNA was dramatically decreased when a lower ipomeanol concentration (5.5 μM) was used. The covalent binding, which is believed to be the initial lesion leading to the pulmonary toxicity resulting from exposure to ipomeanol, was quantitatively similar in perfused lungs to that occurring *in vivo*. This finding supports the hypothesis that the pulmonary toxicity of ipomeanol results from properties of lung tissue *per se* and does not result from transport of reactive metabolites from other organs, such as the liver, to the lungs.

10) The *l*- and *d*- forms of styrene oxide have been synthesized and purified. An HPLC system has been developed which allows the separation of the two-positional isomers formed upon reaction of *l*-styrene oxide with glutathione, but which does not separate the two conjugates formed from *d*-styrene oxide and GSH. The other intermediates formed during mercapturic acid biosynthesis (the cysteinylglycine, cysteine and N-acetylcysteine derivatives) have also been synthesized and characterized.

Proposed Course -- 1) The metabolism of BP will be investigated in the isolated perfused rabbit lung to assess the extent of conjugation by sulfation, glucuronidation, and thioether formation, and to study the effect of inducing and inhibiting agents on the profile of BP metabolites in this system.

2) Further studies on the metabolism of BP in rabbit lung subcellular fractions will be conducted to delineate factors which could affect the amount and activity of the cytochrome P-450 types in tissue preparations.

3) The nature of the release of the BP 4,5-oxide:glutathione conjugate into the circulation medium by perfused rabbit or rat lungs given BP 4,5-oxide will be investigated; if active transport is involved, the metabolism and disposition of this thioether metabolite will be studied in whole animals.

4) The mutagenicity of biliary metabolites of BP or BP 4,5-oxide excreted by rats will be tested in *Salmonella typhimurium* strains in the presence and absence of *in vitro* metabolic activation systems.

4) Glucuronide and sulfate conjugates of BP-4,5-dihydrodiol and styrene glycol will be produced by chemical or biosynthetic means and then characterized. These standards will be used in the studies described below.

6) Glucuronyltransferase and sulfotransferase activities of rabbit lung and rat liver will be characterized *in vitro* using subcellular fractions and BP 4,5- and 7,8-dihydrodiols as substrates.

7) The ability of the isolated perfused rabbit lung and rat liver to conjugate BP 4,5- and 7,8-dihydrodiols and styrene glycol with sulfate and glucuronide will be evaluated.

8) The biochemical aspects of ipomeanol activation will be studied using purified rabbit lung cytochromes P-450_I and P-450_{II}. The possible involvement of prostaglandin synthetase will be investigated.

9) The protective role of GSH against ipomeanol-induced toxicity will be investigated using GSH-depleted livers from diethyl maleate-treated rats. Model in vitro systems may also be used in this study.

10) The histological and overt lesions occurring in pulmonary tissue after exposure of rabbits to p-xylene will be defined. The biochemical factor(s), including the importance of metabolite transport from liver to lung, causing the preferential susceptibility of lungs to toxicity induced by this hydrocarbon will be investigated using isolated perfused rabbit liver and lung preparations.

11) Investigation of the metabolism of p-methylanisole in mammalian systems will be carried out. This compound is closely related chemically to the pulmonary toxin p-xylene and offers sites for three different types of C-hydroxylation reactions (aliphatic, aromatic and O-demethylation) on the same molecule. It will serve as a model substrate to determine the specificity of various MFO systems, including those in rabbit lung, with respect to these reactions.

12) The d and l- forms of styrene oxide will be tested for their mutagenic potential in various Salmonella typhimurium strains, in the presence and absence of epoxide hydrase and the GHS S-transferases. The GSH, N-acetylcysteine, cysteine and cysteinylglycine derivatives of styrene oxide will also be checked for genetic activity.

13) Various GSH S-transferases (from mammalian and fish sources) will be checked for stereoselectivity with d- and l-styrene oxide. Mechanistic studies will also be performed with the l-styrene oxide enantiomer since the two possible positional isomers can be separated by HPLC.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Xenobiotics or their chemically reactive metabolic intermediates (e.g., alkene or arene oxides) are detoxicated by several pathways, principally involving conjugation in liver, lung, gut, and other extrahepatic tissues, and excreted from the animal. The activities of these conjugation and excretory mechanisms may be important factors in determining the susceptibility of organs, or individuals, to environmental agents. The developmental patterns of conjugation pathways active in detoxication are of importance since these may determine the susceptibility of the fetus and neonate to environmental contaminants and may also be related to chemical carcinogenesis and teratogenesis. In the real-life situation one is very rarely exposed to a single xenobiotic; rather, one comes in contact with a large number of synthetic organic chemicals including food additives, drugs, plasticizers, insecticides, hydrocarbons, etc. For this reason investigations concerning interactions between more than one foreign compound are of relevance to environmental health.

PUBLICATIONS

Bornstein, W. A., Chuang, A. H. L., Bresnick, E., Mukhtar, H., and Bend, J. R.: Epoxide hydrase activity in liver nuclei: Hydration of benzo(a)pyrene 4,5-oxide and styrene oxide. Chem.-Biol. Interact. 21: 243-246, 1978.

Mukhtar, H., Philpot, R. M., Lee, I. P., and Bend, J. R.: Developmental aspects of epoxide-metabolizing enzyme activities in adrenals, ovaries, and testes of rat. In: Developmental Toxicology of Energy-Related Pollutants, DOE Symposium Series, 1978, Chap. 47, pp. 89-104.

Mukhtar, H., Elmamlouk, T. H., and Bend, J. R.: trans-Stilbene oxide: An inducer of rat hepatic microsomal and nuclear epoxide hydrase and mixed-function oxidase activities. Chem.-Biol. Interact. 22: 125-137, 1978.

Mukhtar, H., Lee, I. P., Foureman, G. L., and Bend, J. R.: Epoxide-metabolizing enzyme activities in rat testes: Postnatal development and relative activity in interstitial and spermatogenic cell compartments. Chem.-Biol. Interact. 22: 153-165, 1978.

Smith, B. R., Philpot, R. M., and Bend, J. R.: Metabolism of benzo(a)pyrene by the isolated perfused rabbit lung. Drug Metab. Dispos. 6: 425-431, 1978.

Smith, B. R. and Bend, J. R.: Metabolism of benzo(a)pyrene 4,5-oxide by isolated perfused rabbit lung. In Jones, P. W. and Freudenthal, R. K. (Eds.): Polynuclear Aromatic Hydrocarbons, Carcinogenesis. New York, Raven Press, Vol. 3, 1978, pp. 341-346.

Smith, B. R., Maguire, J. H., Ball, L. M., and Bend, J. R.: Pulmonary metabolism of epoxides. Fed. Proc. 37: 2480-2484, 1978.

Bend, J. R., Smith, B. R., Van Anda, J., Ryan, A. J., and Fouts, J. R.: Biotransformation of styrene oxide by the isolated perfused rat liver and by subfractions of homogenized liver cells. In Fouts, J. R. and Gut, I. (Eds.): Industrial and Environmental Xenobiotics: In Vitro versus In Vivo Biotransformation and Toxicity. Amsterdam, Excerpta Medica, 1978, pp. 62-70.

Mukhtar, H., Lee, I. P., and Bend, J. R.: Glutathione S-transferase activities in rat and mouse sperm and human semen. Biochem. Biophys. Res. Commun. 83: 1093-1098, 1978.

Mukhtar, H., Philpot, R. M., and Bend, J. R.: The postnatal development of microsomal epoxide hydrase, cytosolic glutathione S-transferase and mitochondrial and microsomal cytochrome P-450 in adrenals and ovaries of female rats. Drug Metab. Dispos. 6: 577-583, 1978.

Wolf, C. R., Szutowski, M. M., Ball, L. M., and Philpot, R. M.: The rabbit pulmonary mono-oxygenase system: Characteristics and activities of two forms of pulmonary cytochrome P-450. Chemico-Biol. Interact. 21: 29-43, 1978.

Bend, J. R., Smith, B. R., Ball, L. M., and Mukhtar, H.: Arene and alkene oxide metabolism in hepatic and extrahepatic tissues-pharmacological and toxicological aspects. In Aitio, A. (Ed.): Conjugation Reactions in Drug Biotransformation. Amsterdam, Elsevier/North Holland, 1978, pp. 3-16.

Mukhtar, H., Elmamlouk, T. H., Philpot, R. M., and Bend, J. R.: Rat hepatic nuclear cytochrome P-450 and epoxide hydrase in membranes prepared by two methods. Similarities with the microsomal enzymes. Mol. Pharmacol. 15: 192-196, 1979.

Mukhtar, H., Elmamlouk, T. H., and Bend, J. R.: Epoxide hydrase and mixed-function oxidase activities of rat liver nuclear membranes. Arch. Biochem. Biophys. 192: 10-21, 1979.

Aitio, A. and Bend, J. R.: Inhibition of rat liver glutathione S-transferase activity by aprotic solvents. FEBS Letters. In press.

Smith, B. R. and Bend, J. R.: Metabolism and excretion of benzo(a)pyrene 4,5-oxide by the isolated perfused rat liver. Cancer Res. In press.

Smith, B. R., and Bend, J. R.: Metabolism and toxicity of benzo(a)pyrene 4,5-oxide in the isolated perfused rat liver. Toxicol. Appl. Pharmacol. In press.

Mukhtar, H., Leakey, J. E. A., Elmamlouk, T. H., Fouts, J. R., and Bend, J. R.: Precocious development of hepatic glutathione S-transferase activity with glucocorticoid treatment in neonatal rat. Biochem. Pharmacol. In press.

Wolf, C. R., Smith, B. R., Ball, L. M., Serabjit-Singh, C., Bend, J. R., and Philpot, R. M.: The rabbit pulmonary mono-oxygenase systems: Catalytic differences between two purified forms of cytochrome P-450 in the metabolism of benzo(a)pyrene. J. Biol. Chem. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80031-03 LP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Role of Altered Membrane Function in Xenobiotic Toxicity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John B. Pritchard	Research Physiologist	LP NIEHS
	Anthony F. Almeida	Visiting Fellow	LP NIEHS

COOPERATING UNITS (if any)

C. W. Holliday, Mt. Desert Island Biol. Lab
G. J. Neufeld, Emporia State University, Emporia, Kansas 660801

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Comparative Pharmacology and Physiology

INSTITUTE AND LOCATION

NIEHS/NIH/Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Many aquatic animals are highly sensitive to certain xenobiotic compounds. Such organisms are used as models to identify those physiological processes most sensitive to environmental pollutants. Particular emphasis is placed on membrane functions which underlie these processes. We have utilized as a model system the blue crab (Callinectes sapidus) which is extremely sensitive to organochlorine pesticides, particularly at low environmental salinity where its survival depends on its ability to osmoregulate,. The objective is to establish the mechanism(s) responsible for the sensitivity of crustacea to organochlorine compounds. Parameters examined include 1) the levels of Na,K-ATPase in the gills after adaptation to various environmental salinities; 2) the ouabain binding characteristics of the enzyme; 3) the activity of Na,K-ATPase enzyme after administration of DDT both in vitro and in vivo; 4) the mode of inhibition of the enzyme in vitro; and 5) the ability of the intact animal to maintain hemolymph osmolality and ion levels after intravascular administration of DDT.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Na,K-ATPase is routinely measured in freeze-dried, reconstituted homogenates of gill tissue by a modification of the method of Miller et al. (1976). Na,K-ATPase is also partially purified by detergent extraction and differential centrifugation. The coupled, continuous optical assay of Schoner et al. (1967) is used to measure activity of the purified enzyme. Ouabain binding is determined by Millipore filtration. Hemolymph osmolality is measured by vapor pressure osmometry and ion content by flame photometry.

MAJOR FINDINGS AND PROPOSED COURSE: Work this year has focused primarily on (1) characterization of the biochemistry of the blue crab gill Na,K-ATPase and 2) evaluation of the role of this enzyme in osmoregulation by the blue crab. This information is required if we are to determine the mechanism of organochlorine inhibition of this enzyme and the role of altered sodium transport in the toxicity of the organochlorines to this sensitive species. With this data, we will then be able to determine the utility of the Na,K-ATPase model in assessing the membrane toxicity of this and other classes of xenobiotics.

Biochemistry: 1) The affinity of blue crab gill Na,K-ATPase for substrate (ATP) or in cofactors (K) was very similar to mammalian enzymes. 2) Like other membrane-bound enzymes, the gill Na,K-ATPase showed a break in the Arrhenius plot, emphasizing the importance of membrane lipids and temperature in Na,K-ATPase function. 3) The enzyme was much less sensitive to inhibition by either ouabain and vanadium than mammalian Na,K-ATPase (~100 times less sensitive), despite the fact that the gill enzyme was at least as sensitive to DDT (significant inhibition at 0.5 ppm). 4) Ouabain binding to the gill enzyme was also markedly different from mammalian Na,K-ATPase. Its K_D for ouabain was 6.6 μM (vs. 33 nm for dog brain enzyme) and its dissociation was also much more rapid. Many of these effects could be modulated by temperature. Since many of these parameters may be modulated by the lipid composition of the enzyme's local environment, and since lipid also modifies the inhibition by DDT in other systems, we will examine the effect of altering the lipid composition and temperature on ATPase function in vitro. In particular, we will use partial purification of the enzyme to assess the relative roles of its lipid and protein components in organochlorine inhibition. The binding of DDT to purified Na,K-ATPase will be determined and correlated with the degreee of inhibition and the presence of specific lipids. These studies will utilize both mammalian and crustacean Na,K-ATPase.

Osmoregulation: 1) We have recently shown that while gill Na,K-ATPase activity is increased following transfer of the blue crabs to a dilute environment, hemolymph osmolality and sodium reach their new steady-state values prior to Na,K-ATPase. 2) Injection of DDT (0.3 ppm) in vivo produces a transient deficit in osmoregulatory ability. It is therefore very important to determine whether osmoregulation in the blue crabs is primarily mediated by Na,K-ATPase activity or is there second mechanism governing rapid adaptation which is also DDT sensitive. We will therefore focus on identification of the specific trigger for osmoregulatory change and attempt to determine whether DDT also prevents sensing of this signal (for example, sensing of lower Na^+ levels in the environment) in addition to its well-documented inhibition of Na,K-ATPase The second aspect of this study will deal with the response of the blue crab to DDT in the water. The major site of membrane proliferation in the sodium transporting cells of the gill is the apical face of the cell. This implies that most of the Na,K-ATPase sites are located on this face of the cell where they would be in direct contact with the DDT. We will try to verify the anatomical location of the Na,K-ATPase sites via autoradiography using ^3H-ouabain to mark the enzyme sites.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The Na,K-ATPase of crab gill is very sensitive to organochlorine pesticides, is anatomically located in an accessible part of the body, and plays an important role in the homeostasis of this animal. Thus, the blue crab provides an excellent model for the assessment of membrane toxicity of environmental chemicals.

The use of sensitive organisms or organ systems may permit identification of systems particularly prone to disruption by environmental contaminants. Such studies may then a) point to systems which might also be particularly prone to damage in man and b) serve as indicators or warning systems to the accumulation of contaminants in the environment, particularly the marine environment which serves as a sink for persistent pollutants. The sensitivity of the Na,K-ATPase shown here may, in conjunction with the studies proposed above, not only indicate the mechanism of toxicity or organochlorine pesticides in an economically important human food, but it also suggests that the processes linked to Na,K-ATPase activity may be among the earliest and most important affected by DDT.

PUBLICATIONS

Neufeld, G. J., Pritchard, J. B., and Cotton, C. U.: Inhibition of calcium-dependent ATPase from mollusc mantle tissue. Bull. Mt. Desert Island Biol. Lab 17: 56-57, 1977.

Neufeld, G. J., Cotton, C. U., and Pritchard, J. B.: DDT in the rock crab: Tissue distribution, metabolism, and inhibition of gill Na,K-ATPase. Bull. Mt. Desert Island Biol. Lab 17: 75-78, 1977.

Neufeld, G. J. and Pritchard, J. B.: Osmoregulation and gill Na,K-ATPase in the rock crab, Cancer irroratus: Response to DDT. Comp. Biochem. Physiol. 62C: 165-172, 1979.

Neufeld, G. and Pritchard, J.: Biochemical and physiological assessment of DDT toxicity in the blue crab. Proceedings of the Symposium on Aquatic Toxicology, American Society for Testing and Materials, pp. 23-24, 1979.

Pritchard, J. B.: Toxic substances and cell membrane function. Fed. Proc. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80032-03 LP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Excretion of Xenobiotics by Selected Marine and Terrestrial Species

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John B. Pritchard	Research Physiologist	LP NIEHS
	Margaret O. James	Senior Staff Fellow	LP NIEHS

COOPERATING UNITS (if any)
Dept. of Physiology, State University of New York; Mt. Desert Island Biol. Lab, Salsbury Cove, ME; Dept. of Physiology, University of Texas, Medical Branch; Dept. of Medicine, University of North Carolina School of Medicine

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Comparative Pharmacology and Physiology

INSTITUTE AND LOCATION
NIEHS/NIH/Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	0.7	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Marine and terrestrial vertebrates are used to examine the role of organic ion transport in the renal and hepatic excretion of environmental contaminants such as DDT and 2,4-dichlorophenoxyacetic acid (2,4-D). Topics under investigation include examination of 1) the importance of renal and hepatic organic anion transport in the rate of elimination of xenobiotics or their metabolites; 2) the interference of foreign compounds with elimination of endogenous wastes or toxins; 3) the role of intracellular binding proteins, such as glutathione S-transferases, in transport and toxicity of organic ions; and 4) the influence of membrane transport-related cellular accumulation in the development of the xenobiotic toxicity in target organs. The role of transport in the elimination of xenobiotics from specific organs, e.g., brain, as well as from the whole organism, is also characterized.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: In all cases our approach is designed to evaluate the effects of xenobiotics at multiple levels of organization. Thus, we utilize renal clearance determinations *in vivo* in conjunction with isolated renal tubules *in vitro* or purified membrane vesicle preparations to characterize the handling of foreign compounds. In this manner we can characterize the details of interaction with cell membranes and follow the consequences of these interactions to the intact tissue or whole animal. Thus, we hope to avoid the pitfall of placing unwarranted emphasis on an *in vitro* effect before the significance of the observation is evaluated in the intact animal.

MAJOR FINDINGS AND PROPOSED COURSE: 1) Metabolism vs. Transport: Comparison of the excretion rate for anionic xenobiotics with the extent of their metabolism in several marine species revealed an apparent inverse correlation between the extent of metabolism (taurine conjugation) and the rate of elimination. Since it is usually believed that conjugation leads to more water-soluble, more readily excreted metabolites, this was a surprising observation. Using a combination of renal clearance measurements and *in vitro* transport and metabolism studies, it was shown quite clearly that species differences in transport, rather than in metabolism, were the determining factors. Thus, the winter flounder, *Pseudopleuronectes americanus*, excretes 2,4-dichlorophenoxyacetic acid (2,4-D) more rapidly than phenylacetic acid (PA) or phenacetyltaurine (PAT) because the renal organic anion transport system responsible for elimination of the compounds has a much greater affinity for 2,4-D. PA and PAT were transported similarly. Similar results were obtained in the dogfish shark, *Squalus acanthias*. The latter studies were done in collaboration with Dr. F. J. Koschier of SUNY, Buffalo.

2) Effects on Subcellular Organelles: Transport of anionic xenobiotics leads to extensive intracellular accumulation of these compounds, which include 2,4-D, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and 2,2-bis-(*p*-chlorophenyl) acetic acid (DDA), the acetic acid derivative of DDT. The structure of these compounds is similar to known uncouplers of mitochondrial respiration, such as 2,4-dinitrophenol (DNP), with which they also share the transport mechanims. Subcellular distribution studies showed that ~10% of the intracellular DDA was associated with the mitochondria. Oxygen consumption by rat cortical slices showed a biphasic response. There was no change or only a slight increase in O_2 consumption at low DDA concentrations (1 and 10 μM) and significant inhibition of respiration at 100 and 1000 μM DDA. With isolated mitochondria, DDA, 2,4,5-T, and 2,4-D all gave the classical biphasic response seen with DNP. Low concentrations (0.9-10 μM) gave clear-cut uncoupling, whereas higher concentrations also inhibited ADP-stimulated, or State III, respiration. Binding studies (Scatchard) on the isolated mitochondria showed two distinct classes of DDA binding sites. The first had fewer sites (15 nmol/mg mitochondrial protein) and an affinity constant (K) of 4×10^3 M^{-1}. It was blocked by both probenecid and DNP. Total sites for the second class were 177 nmol/mg and a K of 9.6×10^2 M^{-1}. It was only blocked by DNP. Thus, it appears that the low capacity, high affinity site

was associated with the uncoupling, while the second class of sites may represent the functional group associated with the inhibition of State III respiration. Dr. D. U. Silverthorne of the University of Texas Medical Branch collaborated on portions of this work.

3) Choroid Plexus: A second effect of active transport of anionic xenobiotics is demonstrated by our choroid plexus studies. Choroid plexus also possesses the organic anion transport system which functions to transport anionic neurotransmitter metabolites from the brain and cerebrospinal fluid into plasma for subsequent renal excretion. We have shown that the choroid plexus rapidly accumulates 2,4-D and DDA, and that these compounds competitively inhibit transport of the neurotransmitter metabolites. 2,4-D and DDA also inhibit the oxygen consumption of the choroid plexus, an effect which may explain inhibition of organic cation transport by these anions. Recently in collaboration with Dr. Lorcan O'Tauma of the University of North Carolina School of Medicine, we have begun to investigate the effect of these anions (2,4-D and DDA) on living animals using either the single injection or ventriculo-cisternal perfusion techniques. We hope to be able to use this combination of *in vitro* and *in vivo* techniques to examine the effects of a wide range of xenobiotics which may alter choroid plexus transport.

4) Isolated Membrane Vesicles and Carriers: In many cases, it has been difficult to determine the exact nature of a foreign compound's effect on the cell due to the number of possible explanations for the effects. For example, glucose transport is inhibited by mercury, but this could arise from inhibition of ATP production, or of Na,K-ATPase necessary to maintain the Na^+ gradient, or of the glucose carrier itself. The development of techniques permitting the isolation of membranes from specific portions of the cell membrane now permits us to directly examine interactions between the toxin and the carrier. Furthermore, techniques are rapidly evolving which will permit isolation of specific carriers and still allow us to assess their function, i.e., translocation of substrates across membranes, by incorporation of the isolated carrier into liposomes composed of known phospholipids. The potential of these techniques to assess both normal carrier function and toxic effects is very great.

Recently, in collaboration with Dr. David Miller at Mount Desert Island Biological Laboratory, we have adapted these techniques to the isolation of the organic acid carrier from flounder renal tubules. Then the carrier is incorporated into liposomes composed solely of phosphatidylcholine, the first such reconstitution with a pure lipid. We will now use this preparation to examine such basic questions as the role of the carrier and of intracellular binding proteins in organic anion transport. These techniques are also amenable for use in screening for membrane effects. For example, we will use isolated brush border membranes from rat kidney to assess changes in proximal tubule membrane transport after exposure to CD-metalothionine (in collaboration with Dr. Fowler, LOFT).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An understanding of the mechanisms controlling the ability of marine organisms to eliminate the many foreign compounds reaching the marine environment is vital in predicting the hazards of subsequent consumption of the species by man. Furthermore, the use of model preparations such as the isolated flounder renal tubule permits rapid assessment of the interaction of xenobiotics with renal function, or in the case of organic anions, such as 2,4-D, with other similar transport sites in the body. The confirmation of 2,4-D and DDA inhibition of choroid plexus transport of a normal, but toxic, brain metabolite in the rabbit is an excellent example of the predictive value of such a model system from the marine enivornment. Finally, the addition of the ability to study membrane transport in vesicle preparations will now allow us to study the details of the extractions of xenobiotics with membrane transport independent from the effect on other cellular organelles.

PUBLICATIONS

Pritchard, J. B. and Cotton, C. U.: Evaluation of the renal handling of 2,4-D by the winter flounder. Bull. Mt. Desert Island Biol. Lab 17: 71-75, 1977.

Booz, G. W., Pritchard, J. B., and Kleinzeller, A.: The renal clearance of D-mannose in the winter flounder. Bull. Mt. Desert Island Biol. Lab 17: 44-45, 1977.

Pritchard, J. B., Booz, G., and Kleinzeller, A.: Renal sugar transport in the winter flounder: V. Secretion of 2-deoxy-D-galactose. Amer. J. Physiol. 234: F424-431, 1978.

Pritchard, J. B.: Kinetic analysis of the renal handling of 2,2-bis(p-chlorophenyl)acetic acid (DDA) by rat kidney in vitro. J. Pharmacol. Expt. Ther. 205: 9-18, 1978.

Pritchard, J. B. and James, M. O.: Determinants of the renal handling of 2,4-dichlorophenoxyacetic acid. J. Pharmacol. Expt. Ther. 208: 280-286, 1979.

Bend, J. R., James, M. O., and Pritchard, J. B.: Aquatic toxicology. In Guthrie, F. E. and Perry, J. J. (Eds.): Introduction to Environmental Toxicology. Amsterdam, Elsevier/North-Holland. In press.

Booz, G., Goldstein, J., Pritchard, J. B., and Kleinzeller, A.: The handling of D-mannose at the brush border of the kidney of winter flounder, Pseudopleuronectes americanus. Bull. Mt. Desert Island Biol. Lab. In press.

Miller, D. S., Butler, L. A., and Pritchard, J. B.: Incorporation of an organic anion carrier from winter flounder (Pseudopleuronectes americanus) kidney plasma membranes into liposomes. Bull. Mt. Desert Island Biol. Lab. In press.

Pritchard, J. B., Cotton, C. U., James, M. O., Gigiuere, D., and Koschier, F. S.: Role of metabolism and transport in the excretion of phenylacetic acid and 2,4-dichlorophenoxyacetic acid by marine fish. Bull. Mt. Desert Island Biol. Lab. In press.

Guarino, A. M., Miller, D. S., Arnold, S. T., Pritchard, J. B., Davis, R. D., Urbanek, M. A., Miller, T. J., and Litterst, C. L.: Platinate toxicity: Past present and prospects. Cancer Therapeutics Reports Symposium: Pharmacology and Toxicology of Platinate Drugs. In press.

Pritchard, J. B.: Toxic substances and cell membrane function. Fed. Proc. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80036-01 LP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Microsomal Mixed-Function Oxidase System: Protein Chemistry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Shelley R. Slaughter	Staff Fellow	LP NIEHS
Other:	Charles S. Scott	Chemist	LP NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Toxication-Detoxication

INSTITUTE AND LOCATION

NIEHS/NIH/Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Two forms of rabbit pulmonary cytochrome P-450 and a single form of rabbit hepatic phenobarbital-induced cytochrome P-450 have been purified and characterized according to their substrate specificities, spectral properties, and immunological properties. The Form I pulmonary cytochrome and the phenobarbital-induced hepatic cytochrome are similar by the criteria stated above, whereas the Form II pulmonary cytochrome differs from the other two. The objective of this study is to probe the structural relationship between these cytochromes. These relationships are being examined by SDS-polyacrylamide gel electrophoresis, peptide mapping, and N- and C-terminal analyses.

PHS-6040

PROJECT DESCRIPTION

METHODS EMPLOYED: Molecular weight determination by SDS-polyacrylamide gel electrophoresis, peptide mapping utilizing SDS gels, N-terminal and C-terminal analyses were done according to existing methods in the literature.

MAJOR FINDINGS AND PROPOSED COURSE: Purified rabbit pulmonary cytochrome P-450_I (P-450_I) and rabbit hepatic phenobarbital-induced cytochrome P-450 (P-450_{PB}) were shown to have monomeric molecular weights of 52,000 as compared to 58,000 for pulmonary cytochrome P-450_{II} (P-450_{II}). The molecular weights of the cytochromes, as determined by SDS-polyacrylamide gel electrophoresis at a single gel concentration, appear to be valid as judged by the linearity and y-intercept of the Ferguson plot of acrylamide concentration vs. $\log_{10}$ mobility. Molecular weights determined from SDS-gel electrophoresis were consistent with those determined from amino acid analyses. The cytochrome have been mapped by Urea-SDS polyacrylamide gel electrophoresis. P-450_I and P-450_{PB} were shown to have identical patterns whereas the maps of P-450_{II} were significantly different. Protease digestion of P-450_I and P-450_{PB} also produced identical patterns, while such digestion of P-450_{II} differed. Based upon the above results, which are consistent with the spectral, substrate specificity, and immunological similarities of P-450_{PB} and P-450_I and the dissimilarity of P-450_{II}, it is concluded that P-450_{PB} and P-450_I are the same protein or, at least, highly similar proteins, whereas P-450_{II} is a totally different species.

The present work involving the structural studies of the pulmonary and hepatic forms of cytochrome P-450 will be continued and will be expanded to include the rabbit pulmonary and hepatic cytochrome P-450 reductases.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A structural characterization is necessary to the full understanding of the mixed-function oxidase system which is involved in drug interaction and drug metabolism and disposition of inhaled environmental pollutants, as well as pesticide detoxication.

LABORATORY OF PULMONARY FUNCTION AND TOXICOLOGY

THE LABORATORY OF PULMONARY FUNCTION AND TOXICOLOGY
Summary Statement

The purpose of this laboratory is 1) to conduct studies on the structure and function of the normal respiratory tract, 2) to investigate mechanisms of injury and repair in respiratory tract tissues exposed to environmental toxins, 3) to study the pathogenesis of specific disorders of pulmonary tissues (e.g., pulmonary fibrosis). The ultimate goal is to obtain new insights into the pulmonary biology and pathology of the lung which will provide the scientific basis for designing rational measures for prevention of pulmonary diseases. To carry out its mission, the Laboratory of Pulmonary Function and Toxicology must encompass a diversity of scientific talents and interests including biochemistry, cell biology, immunology, toxicology, and pathology. Presently the LPFT consists of the following groups: endocrinology, prostaglandins, biochemical pathology, cell biology, structural pathology, and environmental carcinogenesis.

BACKGROUND AND SCOPE

The respiratory tract is the organ system, bearing the heaviest burden of environmental contamination brought about by urbanization and industrialization. It is the target for numerous gaseous and particulate air pollutants originating from automobiles and industries. In addition, intense occupational exposure of the lungs occur in a wide variety of work environments. We have also learned in recent years that substances entering the body by routes other than the airways (e.g. ingestion of food additives, parenteral administration of pharmaceuticals), may cause severe toxic changes of respiratory tract tissues. As a result of the great variety of exposures (not all of them man-made), acute and chronic respiratory disorders are among the most common diseases with environmental factors as major etiological components. Yet our understanding of the pathogenesis of even the major groups of chronic respiratory diseases: bronchitis, emphysema, pulmonary fibrosis, and bronchogenic carcinoma is still rudimentary. At present, we are neither able to detect these diseases at an early stage of development, when the damage is presumably still reversible, nor are we able in the majority of cases to halt their progression effectively, once the diseases are diagnosed. The reasons for this dilemma are many. One is the often uncertain and apparently complex etiology with multiple interacting factors (individual susceptibility, predisposing injuries or diseases, combination of insults); another is the inadequacy of our basic knowledge of pulmonary biology. The respiratory tract is a complex organ system composed of multiple segments with a great number of cells with diverse functions, many of which are only vaguely understood. This complexity has made it difficult to investigate the respiratory and non-respiratory functions of the various lung tissues and cells, their unique metabolic characteristics, their life cycle and turnover, their susceptibility to injury, and their capacity for repair. It is clearly evident that new experimental approaches have to be developed to advance our knowledge in the field of pulmonary biology and pathobiology.

Studies in the pulmonary branch will be conducted at various levels of biological organization. The major emphasis, however, will be concentrated at the cellular and biochemical level. The research efforts will encompass studies of normal cell and tissue functions as well as their perturbations by various environmental toxic agents.

PULMONARY ENDOCRINOLOGY

The research efforts in this program are designed to provide information on those cells of the lung which are considered to have endocrine or neuroendocrine functions. The origin, innervation and bioactive components of lung endocrine cells are explored and the hormonal activity of various lung neoplasms is being studied. Attempts to identify polypeptide hormones in the neuroendocrine cells of the guinea pig trachea were conducted with more than 20 highly specific antisera to polypeptide hormones such as substance P, bradykinin, neurotensin and others. No immunoreactivity was detected raising the possibility that the neuroendocrine cells of the airways contain a heretofore unidentified polypeptide hormone.

Reports from the literature suggested that the carcinogen diethylnitrosamine (DEN) causes significant cellular alterations of the neuroepithelial bodies in the lungs of hamsters. Experiments stimulated by these reports showed that DEN increases the number and size of the neuroepithelial bodies in hamsters. Cell cultures prepared from DEN exposed hamster lungs showed large numbers of cells containing dense cored vesicles some of which stained with corticotropin antisera, suggesting that the cells have been induced by DEN to produce ACTH.

In a separate study squamous cell carcinomas induced in rat lungs by carcinogenic polycyclic hydrocarbons were studied for immunoreactivity to ACTH. Both the in vivo tumor lines as well as the in vitro cell lines derived from such tumors were found to contain low levels of ACTH. Studies with mast cells have shown that these cells contain sequences of ACTH and β-lipotropin. The biosynthetic activity of normal and neoplastic mast cells is being investigated.

PROSTAGLANDIN RESEARCH

Prostaglandins (PG's) and thromboxanes (TX's) are a group of acidic lipids derived from the unsaturated fatty acid, arachidonic acid (AA). These lipids are ubiquitous to all mammalian cells. The levels of PG's and TX's in most tissues are low; but in response to a variety of stimuli, PG's and TX's are rapidly synthesized and are subsequently metabolized. Most PG's and TX's are, therefore, short-lived chemical mediators of biological processes. PG's and related substances have an extremely diverse biological activity. They are implicated in the control of renal function, vasomotor tone bronchomotor tone, reproduction, and as mediators of inflammation. Recent developments indicate that a balance between two PG's, one formed by blood vessels and one formed by platelets, controls intra-arterial thrombosis.

Recent findings have also shown that PG's may be involved in promotion of tumor formation by a number of chemical agents. Our efforts are focused on studies of PG and TX biosynthesis and metabolism in the lung since this organ is particularly active in the synthesis and inactivation of these fatty acid derivatives.

Through structural activity studies, the moieties critical for the transport of the PG into the lungs were determined (acid group at C-1, oxygen group at C-1, hydroxyl group at C-15 in the S-configuration). Experiments with PGI_2, a potent dilator of blood vessels and inhibitor of platelet aggregation suggested that the lung is probably not an essential site for its metabolism. Metabolic studies with thromboxane A_2 have clarified essential features of its enzymic breakdown to HHT and as yet unidentified metabolite. The regulation of the biosynthesis of prostacyclin and other PG's is studied in a variety of cell lines. Also investigated are mechanisms by which Prostaglandins might be involved in carcinogenesis. These studies include investigations of the co-oxygenation of benzo(a)pyrene during the metabolism of arachidonic acid as well as experiments to explore the possible role of prostaglandins in tumor promotion.

BIOCHEMICAL PATHOLOGY

The research efforts of this group are concerned with the biochemistry of the acellular lining layer of the terminal airways in health and disease. Our goal is to identify major protein constituents in the acellular lining of the lungs of normal individuals and to determine whether significant alterations in the composition of the extracellular alveolar lining occur in various pulmonary diseases such as alveolar proteinosis. Recently two proteins with molecular weights of 47,000 and 52,000 daltons were found in lavage effluents from patients with pulmonary alveolar proteinosis. These proteins may be diagnostic for this disease.

Other studies are concerned with the identification and origin of hydrolases in the extracellular lining of the alveoli. These studies suggest that the lamellar bodies which are secreted by type II alveolar cells onto the alveolar surface are probably modified lysosomes. The biochemical processes involved in the formation of lamellar bodies are being investigated. Evidence was presented suggesting that the transfer of phosphatidylcholine and phosphatidylinositol from microsomes, mitochrondria and synthetic liposomes to lamellar bodies is facilitated by transport proteins obtained from pulmonary cytosol.

PULMONARY PATHOLOGY

One major element of this prpgram is concerned with the mechanism of toxicity of inhaled particles and fibers. Chrysotile asbestos is composed primarily of magnesium hydroxide (Mg) and Silicon dioxide (Si). Its toxicity has been linked to its Mg content because magnesium-leached fibers have shown reduced toxicity under a variety of circumstances. The initial studies in this program are aimed at establishing the proper procedures to measure Mg and Si in native asbestos fibers, and in fibers embedded in cells or tissues, using electron microprobe analysis. It was demonstrated that the Mg/Si ratio's can be reproducibly established in single fibers. The ratio was shown to change significantly in fibers exposed _in vitro_ to 1N HCl and _in vivo_ three months after fibers had been deposited in various tissue compartments in the lungs of rats. Studies on the deposition and translocation of inhaled asbestos are underway. Early results indicate that fibers deposited in the terminal airways probably reach the lung interstitium by way of intra as well as inter-epithelial particle transport.

Studies of the conducting airways have focused on the non-ciliated cell (Clara cell) in the distal airways. Comparison was made on the ultrastructural level between Clara cells from eleven different species, including man. These studies suggest the existence of three different types of Clara cells depending on the relative abundance of secretory granules and agranular endoplasmic reticulum. It is proposed that these ultrastructural types correspond to three functional types: predominantly secretory; predominantly metabolically active; (drug metabolism); and a combination of both. Studies are planned to test this hypothesis.

PULMONARY CELL BIOLOGY

The goal of this program is to study the life cycle and function of selected lung cell populations. Major emphasis is placed on developing the means to investigate epithelial cells from the conducting airways _in vitro_. Culture conditions are being established which allow us to study the mechanisms of epithelial cell differentiation and the regulation of biosynthesis of specific cell products. Experiments with tracheal epithelial cells from rabbits and rats showed that primary cultures, using conventional medium containing 5-10% serum, deteriorate rapidly losing most of their differentiated features within 2-3 days of culture. After a few weeks of maintenance, the cultures also lose their replicative capacity and die out. It was found, however, that the cultures can be maintained for many weeks if the serum level is drastically reduced and insulin, transferrin and epidermal growth factor are added to the medium. Factors regulating proliferation and differentiation are now being studied in these long-term primary cultures.

ENVIRONMENTAL CARCINOGENESIS

This research effort has two major goals: To study mechanisms involved in respiratory carcinogenesis and to investigate mechanisms of "neoplastic progression."

In vivo studies using the heterotopic tracheal transplant system have produced new information regarding the fate of metaplastic-dysplastic lesions commonly considered morphological indicators of neoplastic development in respiratory tract epithelium. It was shown that many of the lesions induced by a carcinogenic insult of short duration and moderate severity (producing a 10% incidence of invasive carcinomas) are reversible and most of them do _not_ progress to invasive cancer. This finding is being further analyzed using _in vitro_ methods to study neoplastic progression.

In a separate line of research, _in vitro_ systems were developed to study neoplastic transformation and promotion _in vitro_ using tracheal epithelial cells. Using these systems, it was shown that tracheal epithelium in various states of organization i.e., organ cultures, primary cultures, epithelial cell lines, can effectively metabolize benzo(a)pyrene (BaP) to its major metabolites. Preliminary evidence also suggests that neoplastic transformation also occurs following exposure to BaP and some of its metabolites.

In vitro studies are also carried out with hamster epidermal cells specifically to study the relationship between the regulation of cellular differentiation and carcinogenesis. A main focus of study is the role of somatic mutation in neoplastic progression. One model system used is the spontaneous transformation of Syrian hamster embryo cells in culture which occurs in at least two steps, an aneuploid preneoplastic state and a neoplastic state characterized *in vitro* by a stable phenotype, namely, anchorage independent growth. Various studies suggest that the appearance of this phenotype has a mutational basis. Fusion studies with diploid hamster embryo cells indicate that anchorage independence possibly is a recessive characteristic of transformed cells.

Other systems are also being developed to study neoplastic progression in epithelial cell systems particularly the progression of benign to malignant tumors. In this context methods to study the phenotype of invasiveness are being established.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80033-03

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Synthesis of Polypeptide Hormones and Prohormones by Normal and Neoplastic Lung Epithelium

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. P. DiAugustine	Head, Endocrinology Group	LPFT	NIEHS
	L. H. Lazarus	Research Chemist	LPFT	NIEHS
Others:	M. Khan	Visiting Fellow	LPFT	NIEHS
	I. Linnoila	Visiting Associate	LPFT	NIEHS
	P. Nettesheim	Chief, LPFT	LPFT	NIEHS
	E. Sisskin	Staff Fellow	LPFT	NIEHS
	J. C. Barrett	Senior Staff Fellow	LPFT	NIEHS
	M. D. Erisman	Post-doctoral Fellow	Duke Univ.	

COOPERATING UNITS (if any)

Pulmonary Carcinogenesis Program, Biology Division, Oak Ridge National Laboratory

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Endocrinology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 5.0	PROFESSIONAL: 4.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The lung epithelium of mammals is known to contain neuroendocrine-like cells and neuroepithelial bodies. Dense-cored or neuroendocrine-type granules have been previously identified in both of these structures. We are attempting to identify known polypeptides hormones in the normal and neoplastic lung epithelium of various species. Although no corticotropin immunoreactivity was identified in normal lungs by immunohistochemical methods, this hormone was identified in cultured cells obtained from hamsters treated with diethylnitrosamine. These cells were also argyrophilic and contained numerous dense-cored vesicles, properties observed in human bronchial carcinoids and in some human small cell carcinomas. These results contrast with those obtained with chemically-induced rodent squamous cell carcinomas. Such tumors exhibited neither immunohistochemical evidence for corticotropin nor cells containing dense-cored vesicles. We are presently investigating the regeneration of tracheal epithelial cells in order to identify pluripotent or progenitor cells for lung neuroendocrine cells and related neoplasias.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: General histological procedures were used for fixation and staining of tissue sections for light and electron microscopy. Fluorescent antibodies and the immunoperoxidase bridge method were used for the cytochemical localization of polypeptide hormones. Cell culture, radioimmunoassay, thin-layer and column chromatography, and sodium dodecyl sulfate gel electrophoresis are being used in the studies described below. Radioisotopes are measured with a gamma or liquid scintillation spectrometer.

MAJOR FINDINGS AND PROPOSED COURSE: The present program consists of investigations of putative neuroendocrine cells of the pulmonary epithelium and related neoplasias.

1. Neuroendocrine cells of the pulmonary epithelium. It is well known that the lungs of mammals contain singular epithelial cells or K cells, which have neurosecretory-type dense-cored vesicles. These cells are generally argyrophilic and demonstrate characteristic amine fluorescence following administration of precursors such as 5-hydroxytryptophan. We examined the neuroendocrine-like cells of the guinea pig trachea for immunoreactivity with antisera to known polypeptide hormones. Antiserum to substance P, bradykinin, neurotensin, vasoactive intestinal peptide and other known polypeptide hormones did not exhibit immunostaining. It is possible that K cells contain a bioactive polypeptide hormone heretofore not identified.

Tracheal segments transplanted subcutaneously into recipient inbred guinea pigs regenerated a mucociliary epithelium. The frequency and appearance of the K cells were not different than that observed in the trachea of the recipient animal. One purpose of this experiment was to observe any regeneration of K cells in the absence of apparent tracheal innervation. Occasionally we have observed beaded nerve fibers in sections of guinea pig trachea stained for argyrophilia. These fibers were observed in the submucosa and appeared to make contact with the basal region of the K cell. We are presently attempting to also identify these fibers in the transplanted trachea.

We also propose to further investigate the regeneration of K cells by using epithelial cell lines derived from the trachea. The formation of a mucociliary epithelium will be studied in transplanted tracheas previously treated to destroy mitotic cell populations. We shall attempt to select cell lines containing no evidence of K cells using histochemical methods. The regeneration of a tracheal mucociliary epithelium containing K cells should indicate that these cells are not progenitor cells.

2. Identification of a lung polypeptide hormone. Human bronchial carcinoid tumors have been reported to have a relatively high concentration of known polypeptides, exceeding in many cases 100 μg per gram wet weight. We shall attempt to grow these human tumors in athymic nude mice in order to obtain sufficient tissue for isolation of peptides with a molecular weight less than

10,000 daltons. Use of ^{3}H-amino acids of high specific activity in incorporation studies should enable identification of peptides separated by thin-layer and column chromatography and two-dimensional gel electrophoresis. Since most endocrine tumors still produce the polypeptide hormone associated with the tissue from which the tumor originated, we propose that human bronchial carcinoids should be an appropriate source for isolating bioactive polypeptides associated with lung neuroendocrine cells.

3. Chemical induction of lung cells containing corticotropin. The carcinogen diethylnitrosamine (DEN) has been previously reported to stimulate proliferation of lung neuroendocrine cells of the hamster. In the present study, three mg of DEN was injected subcutaneously twice a week into hamsters. The treated animals revealed an apparent increase in size and number of neuroepithelial bodies. Lung cells of treated and control animals were disassociated by treatment with 0.1% pronase and cultured for one week in F-12 media containing 10% fetal calf serum. The cultures from two trials made with treated animals contained fibroblasts and cells with dense-cored (neruosecretory-type) vesicles. These cells were also argyrophilic and could be immunostained with corticotropin antisera. Essentially only fibroblasts were observed in cultures of cells obtained from control hamsters treated with a saline solution.

Thus, we have shown that a carcinogen can induce lung cells with properties of small cell carcinoma. We postulate that the endocrine-like cells observed in vitro were derived from neoplasias of the neuroepithelial body. In further studies, we shall attempt to obtain cloned cell lines and transplantable tumors from treated animals and use these for various studies, such as the origin, pluripotent capacity, and effect of various growth factors on lung endocrine-like neoplasias.

4. Polypeptide hormones in chemically-induced squamous cell carcinomas and cloned subpopulations. Extracts of chemically-induced rodent lung squamous cell carcinomas were measured for corticotropin by radioimmunoassay. The low levels (7-20 ng ACTH/gm wet weight tumor) found correlate well with the fact that these tumors have few, if any, neurosecretory-type granules and that patients with this histological class of lung tumors usually do not develop Cushing's syndrome or other endocrinopathies. Gel filtration of extracts using Sepharose CL-6B with 4M urea revealed a large molecular weight form of ACTH >20,000 daltons and a smaller form with an elution volume identical with that of ^{125}I-ACTH$^{1-39}$. A low level of "processing enzymes" might allow precursor forms of hormones to exist in certain classes of tumors. We are presently examining extracts for growth hormone-immunolike activity.

Preliminary results from in vitro studies also indicate that colonies with distinct morphological and growth properties can be obtained in cultures of cells derived from a chemically-induced lung tumor. ACTH immunoreactivity appears to be clearly a product of squamous carcinoma cells, although it is possible that these cells can be pluripotent and form specific cells with endocrine-like properties. However, all the clones heretofore derived *in vitro* from one rodent squamous cell carcinoma were found to contain ACTH immunoreactivity at nearly identical levels, which argues against a separate population of cells being active in hormone synthesis.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Polypeptide hormones secreted by endocrine organs are known to have specific physiological effects at very low serum concentrations. Numerous clinical disorders are known to be due to abnormal levels of endocrine secretions or complementary receptors. Identification of polypeptide hormones and proteins produced by normal lung epithelial cells is important for understanding the factors which act to regulate vital lung functions. Measurement of these peptides in biological fluids could be important in the detection of early stages of lung neoplasias or in the assessment of chemotherapeutic treatment of this disease.

PUBLICATIONS

Linnoila, R. I., DiAugustine, R. P., Miller, R. J., Chang, K. J. and Cuatrecasas, P.: An Immunohistochemical and Radioimmunological Study of the Distribution of [Met^5]- and [Leu^5]-Enkephalin in the Gastrointestinal Tract. Neuroscience 3: 1187-1196, 1978.

Linnoila, I., Abe, T., Voytek, P. and DiAugustine, R. P.: Coupling of Amines to and Cross-linking of Endogenous Cytosol or Membrane Proteins by Hepatic Transglutaminase. *Biochem. Pharmacol.* In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25005-02 LPFT

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Synthesis and Release of Polypeptide Hormones by Mast Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. H. Lazarus	Research Chemist	LPFT NIEHS
Other:	R. P. DiAugustine	Head, Endocrinology Group	LPFT NIEHS
	R. I. Linnoila	Visiting Associate	LPFT NIEHS
	M. N. Khan	Visiting Fellow	LPFT NIEHS
	R. Wu	IPA	LPFT NIEHS
	G. Jahnke	Chemist	LPFT NIEHS
	M. D. Erisman	Postdoctoral Fellow	Duke University & LPFT NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Endocrinology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mast cells are ubiquitously distributed throughout mammalian organs in association with connective tissue. Special consideration is being given to the role of mast cells in the lung where these cells are present beneath the epithelium in trachea and bronchi as well as in mucous lining. Mast cells contain bioactive amines, such as histamine and serotonin, sulfonated glycosaminoglycans, e.g., heparin, and peptide chemotactic factors as well as polypeptide hormones such as vasoactive intestinal polypeptide, corticotropin (ACTH) and β-Lipotropin (β-LPH). This investigation then focuses on: (1) the characterization of the precursor of mast cell ACTH and its comparison to that from pituitary glands; (2) the in vitro synthesis of peptide hormones in mast cells; (3) the processing and release of ACTH and β-LPH by bioactive agents and peptide hormones; and (4) the use of a mastocytoma cell line to study the effect of reversible granulopoisis on the synthesis, storage and release of an enkephalin precursor. Studies using a mouse mastocytoma have shown that this tumor contains numerous polypeptide hormones. Therefore, it appears to have the capacity of ectopic synthesis of peptide hormones analogous to that observed in various pulmonary tumors.

PROJECT DESCRIPTION

METHODS EMPLOYED: Mast cells (MC) are obtained from exsanguinated rats by lavage of both the peritoneal and thoracic cavities with a balanced salt solution. The mastocytoma (P-815) is propagated in the peritoneal cavity of mice (CD_2F1) as an ascites fluid. A cell line from the mastocytoma will be established to grow as a suspension culture in order to facilitate studies on the processing and synthesis of peptide hormones. MC will be maintained in *in vitro* culture conditions by use of feeder layers prepared from rat embryos. Mast cells are purified by means of density gradients.

Iodination of peptide hormones utilizes chloramine T as the oxidant and the labeled hormone is isolated free of reactants by passage over gel filtration columns. Quantitation of peptide hormone routinely uses either radioimmunoassay (RIA) techniques or by assessment of biological activity; e.g., the ACTH-mediated release of glucocorticoids from adrenal cell suspensions.

The synthesis, processing and secretion of peptide hormones will entail the use of pulse labelling/chase experiments in cell cultures. The isolation and identification of these nascent peptides will employ such methods as affinity chromatography, immunoprecipitation, molecular seiving, and polyacrylamide gel electrophoresis in combination with electrofocusing. Isolated peptides will be analyzed for their amino acid composition, peptide and sequence. The *in situ* localization of peptide hormones will be accomplished by use of immunohistochemical (IHC) techniques with the lactoperoxidase-bridge method. *In vitro* protein synthesis of peptide hormone precursor(s) will use known procedures.

Granulopoisis in the mastocytoma will be induced by agents known to produce granules and bind to tubulin molecules: butyrate, colchesine and vinblastine. Histamine, serotonin and heparin will be quantitated by published procedures.

MAJOR FINDINGS AND PROPOSED COURSE: (1) *Presence of sequences of ACTH and β-LPH in mast cells:* Antibodies raised against the 16K region (amino terminal half of ProACTH or 31K precursor) of ProACTH, ACTH$^{1-24}$, β-LPH$^{39-45}$, and β-endorphin all stained MC granules by IHC; the immunostaining of ACTH and β-LPH could be competed out by the appropriate peptide. Although substance P and bradykinin release histamine from MC and neurotensin sterospecifically binds to MC membranes, antibodies raised against these peptides failed to stain MC by IHC. Extraction of purified MC and solubilization of the lyophilized extracts yielded parallel displacement curves in the RIA for β-LPH; the ACTH RIA gave variable results from one experiment to another. However, separation of ACTH from a possible contaminant(s) in the MC extract by silic acid showed parallel response in RIAs. Heparin, a sulfated proteoglycan, which is found associated with MC granules, brought about a marked inhibition of the ACTH RIA. Model studies demonstrated that heparin was readily soluble under the conditions employed for the extraction of MC peptides and may be responsible for the problem cited above. The release of ACTH and β-LPH, which is the precursor to β-endorphin and other opiate-like peptides, are in progress: the noncytotoxic degranulation of MC will measure histamine or serotonin release as internal controls. A variety of substances, known to release degranulation remains an important question to be solved. Likewise, we plan to determine whether resynthesis of ProACTH occurs following degranulation of MC plated on feeder cells in in vitro culture conditions or if it represents a pinocytotic uptake of preformed hormone from tissue or blood in the intact animal.

(2) Biosynthesis of ProEnkephalin and Polypeptide Hormones: The presence of a ProEnkephalin in mastocytoma which cross-reacts with a [Leu^5]-enkephalin antibody represents a major finding: although present in nerves and brain, two possible precursors have just been isolated and partially sequenced. We found that ProEnkephalin could be separated into 2 major molecular regions by gel filtration: one that eluted between 4-8,000 daltons and another at approximately 2,450 daltons. This latter material was partially degraded by trypsin, yielding a product of 1,250 daltons, and by CNBr to approximately 1,000 daltons. No material was detected at the elution position of the free enkephalin pentapeptide. The existence of sugar moities will be assessed once sufficient quantities are available. Mice, infected with the mastocytoma, have been exposed to a mixture of tritiated amino acids and the final extract is being immunoprecipitated with numerous antibodies. These immunopreciptates will be analyzed by polyacrylamide electrophoresis. The ability of butryrate to reversibly induce granule formation in these cells will be used in the study of the synthesis, storage, processing and secretion of peptide hormones. The existence of a precursor to the enkephalins in a mastocytoma cell line will offer us a unique opportunity to follow the development of potential neuroendocrine functions in a non-endocrine cell. The isolation of mRNA and its translation in the wheat germ in vitro protein synthesizing system will enable us to determine the true size of the protein backbone of this prohormone.

(3) Binding of Peptide Hormones: The stereospecific binding of neurotensin to MC membranes is well documented. Although ACTH, substance P and bradykinin are known to interact with MC, causing a release of biologically active amines, their direct interaction with membranes has not yet been demonstrated. The possibility exists that even though ACTH is excreted in MC, it may also be involved in a short-loop feedback control affecting the secretion not only of ACTH but other compounds.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is well documented that the principle site of histamine storage is the MC, or in the case of blood, the basophil. MC which are found in pulmonary epithelium or in the submucosa of bronchi and bronchioles, could ready release not only histamine but polypeptide hormones during immune reactions occurring with airborne antigens, the contraction of bronchiolar muscle and constriction of pulmonary arteries are mediated by histamine. The presence of ACTH and opiate peptides in pulmonary tissue could modify or modulate these effects as well as producing their own specific and localized changes: e.g., β-endorphin is one of the most potent natural morphomimetic peptides known, producing marked analgesic reactions in addition to a naloxane-reversible catatonic state. These responses could be regulated through specific cell surface receptors of MC with the release or inhibition of secretion of other humoral substances. A study into the many biological properties of MC should provide a rationale for understanding some of the factors involved in allergic or environmentally-induced pulmonary responses and disease states. Of these, some pulmonary carcinomas resemble the mastocytoma both morphologically and in their ability to ectopically produce bioactive peptide hormones. The mastocytoma can then serve as an in vitro model system for studying the basis of several physiological symptoms that appear during the progression of pulmonary cancer.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80008-05

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Biosynthesis, Release, Transport and Metabolism of Prostaglandins [PGs] in Lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. E. Eling	Head, Prostaglandin Group	LPFT	NIEHS
Other:	K. Sivarajah	Visiting Fellow	LPFT	NIEHS
	R. Warnock	Intermittent	LPFT	NIEHS
	J. Boyd	Temporary Technician	LPFT	NIEHS

COOPERATING UNITS (if any)
Inhalation Toxicology Section, Environmental Biology and Chemistry Branch
Duke University

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Prostaglandin Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.05	PROFESSIONAL: .3	OTHER: .75

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long-range goal of this project to study the inactivation of prostaglandins by the lungs and other tissues. The effects of environmental agents on the pulmonary transport and metabolism of prostaglandins is being investigated, and these effects are being related to pulmonary toxicity and damage caused by the environmental agents. Topics of current interest are the structural requirements of a prostaglandin needed for transport to intracellular spaces, the effect of oxidant gases on transport, and the potentiation of inflammatory reaction by inhibition of pulmonary inactivation of prostaglandins. Biosynthesis of Prostaglandin and Thromboxanes in lung tissue and effect of exposure to environmental agents on biosynthesis and release of PG from lung are also being studied.

PROJECT DESCRIPTION

METHODS EMPLOYED: Prostaglandin (PG) and thromboxane (TX) synthetase activity was measured *in vitro* using the microsomal protein from a variety of tissues and organs as an enzyme source. ^{14}C-Arachidonic acid (AA) or prostaglandin endoperoxides were incubated at 37°C for various times and under several conditions. After incubation, the PG and TX were removed by solvent extraction, separated by thin-layer chromatography or high pressure liquid chromatography, and estimated by liquid scintillation techniques.

An isolated perfused rat, guinea pig, or rabbit lung was used to examine the uptake, metabolism, and efflux of prostaglandins (PGs) and their metabolites from lung tissue. The isolated perfused lung was designed to permit infusion of a constant concentration of PGs and perfusion with drug-free perfusate. PG metabolites were isolated from the perfusate by extraction and separated by thin-layer chromatography techniques. The unidirectional flux of PG into the lung was measured by extrapolation of the net uptake velocity of PG to zero time.

PG and TX were also measured in incubation mixtures or in the perfusate of lung by radioimmunoassays.

MAJOR FINDINGS AND PROPOSED COURSE: PGs are accumulated into lung tissue by a carrier-mediated process and are subsequently degraded by pulmonary enzymes to either 15-keto PG or 15-keto-13,14-dihydro PG. The uptake, metabolism and subsequent release of parent PG and metabolites are remarkably efficient and rapid.

Structural activity studies were done to determine the critical moities of the PG essential for transport into lung tissue. From these studies we have concluded that there are three critical portions of the PG molecules necessary for transport into lung tissue:
(1) acid group at C-1
(2) oxygen function, particularly a hydroxyl group at C-1
(3) a hydroxyl group at C-15 in the S configuration.

Small changes in the geometric relationship of these three portions can greatly alter the lung's affinity to transport and metabolize a PG. For example, conversion of the 15 hydroxyl from an S to R configuration greatly reduces transport. Although we could not elucidate what is responsible for the substrate specificity, it is possible that the critical regions are necessary for the binding of a PG to a carrier molecule.

Recently, an additional PG, called PGI_2, was discovered by Vane and his co-workers. PGI_2 was shown to be a potent dilator of blood vessels and an inhibitor of platelet aggregation. It was proposed that a balance between PGI_2 and thromboxane A_2 could be of importance in cardio-vascular diseases. PGI_2 contains an additional ring formed between the carbon 9 oxygen and carbon 5 on the α side chain. This ring opens in aqueous solution resulting in the formation of 6-oxo-$PGF_{1\alpha}$. Although PGI_2 contains the 3 critical moities required for transport, the α chain and thus the acid group have a different geometric configuration compared compared to the rest of the PG molecule. PGI_2 was rapidly degraded by pulmonary enzymes (in vitro) and appeared to be an excellent substrate for these enzymes. 6-oxo $PGF_{1\alpha}$ was also metabolized but was a poor substrate. However, on passage through the isolated perfused rat lung, neither PGI_2 nor 6-oxo- $PGF_{1\alpha}$ was metabolized. This serves as an additional example of the importance of the transport system in determining the pulmonary metabolism of PG. In contrast to other classical PG, pulmonary metabolism appears not to be the cricital determinant of the in vivo half-life of PGI_2. These studies also indicate that an additional critical region is required for transport. A ring structure formed between carbon 6 and 9 is present in PGI_2 and 6-keto $PGF_{1\alpha}$. This ring alters the geometric relationship of the α side chain to the rest of the PG molecule. In addition, the oxygen function at C-9 is no longer free. Thus a free oxygen function at C-9 or precise geometric relation of the α side chain is required for transport.

We have also studied the effect of exposure of rats and guinea pigs to 100% O_2 and NO_2 and PG biosynthesis and metabolism by lung. O_2 and NO_2 significantly depresses the enzyme system that metabolized PG to inactive metabolites (Prostaglandin dehydrogenase PDGH) but is without effect on prostaglandin synthetase or the transport carriers. After exposure to either NO_2 or O_2, depressed PGDH returned to normal levels within 5 days. Kinetic studies indicate noncompetitive inhibition of PGDH on exposure to O_2 which is consistent with destrutcion of the enzyme by O_2 gas.

We have investigated the potentiaion of anaphylaxis in guinea pig by exposure to 100% O_2. Guinea pigs sensitized to albumin were exposed to 100% O_2 for 72 hrs, the lung isolated and perfused. The perfusate during anaphylaxis was collected and analyzed for vasoactive material. The major prostaglandin (60%) released during anaphylaxis was TXB_2 with lesser amounts of $PGF_{2\alpha}$, PGE_2, 15-keto-13,14 dihydro $PGF_{2\alpha}$. These PG were detected and estimated by RIA. Exposure to 100% increased the TXB_2 release 2-3 times, elevated $PGF_{2\alpha}$, PGE_2 and 6-keto $PGF_{1\alpha}$ release but decreased the release of the $PGF_{2\alpha}$ metabolite 15-keto 13,14-dihydro $PGF_{2\alpha}$. Histamine release, which is also an indication of the degree of anaphylaxis, was unchanged. The exposure to 100% O_2 appears to potentiate the release of TX and PG during anaphylaxis by inhibition of their metabolism. This may be inpart responsible for the potentiation of systemic anaphylaxis observed after exposure to O_2 and other oxidant gases. The proposed course is as follows: 1) To examine the effect of exposure of rats to airborne pollutants on PG systems; 2) To use isolated cell systems to examine the mechanisms for the transport of PG into and out of cells and apply the information obtained from this study to the intact lung system; 3) To examine the effect of exposure of animals to pollutants on the inflammatory release of vasoactive chemicals from the lung; 4) To study prostaglandin biosynthesis and metabolism in human lung; 5) To measure the PG and TX obtained in sample of lavages from human lung with pulmonary disease and attempt to correlate these findings with the disease; and 6) To study PG and TX biosynthesis in isolated lung cells.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: PGs have a large diversity of physiological effects. Alterations in PG control of cellular events may be related to transport of PGs across cell membranes, The lung is an important site for the synthesis and metabolism of PGs; alterations in the PG biosynthesis, release, transport and metabolism systems may be related to toxic effects of exposures to pollutants and induction of lung diseases. The lung makes a variety of PG and TX, but little is known of cells responsible for biosynthesis. This information appears to be necessary to elucidate the role of PG in pulmonary disease.

PUBLICATIONS

Anderson, M. W. and Eling, T. E.: Prostaglandin removal and metabolism by isolated perfused rat lung. Prostaglandins 11: 645-677, 1976.

Eling, T. E. and Anderson, M. W.: Studies on the biosynthesis, metabolism, and transport of prostaglandins by the lung. Agents and Actions 6: 543-546, 1976.

Crutchley, D. J., Eling, T. E., and Anderson, M. W.: ADPase activity of isolated perfused rat lung. Life Sci. 22: 14-3-1420, 1978.

Anderson, M. W., Chaudhari, A., Crutchley, D., Wilson, A. G. E., and Eling, T. E.: Studies on the covalent binding of an intermediate(s) in prostaglandin biosynthesis to various tissue proteins. Biochim. Biophys. Acta. In press.

Chaudhari, A., Eling, T. E., and Anderson, M. W.: Inhibition of pulmonary prostaglandin metabolism by exposure of animals to oxygen or nitrogen dioxide. Biochemistry J. In press.

Chaudhari, A., Anderson, M. W., and Eling, T. E.: Conjugation of 15-keto prostaglandins by glutathione 5-transferase. Biochem. Biophys. Acta. 531: 56-64, 1978.

Hawkins, H. S., Smith, B. J., Nicolaou, K. C., and Eling, T. E.: Studies on the mechanisms involved in the fate of prostacyclin (PGI_2) and 6-keto $PGF_{1\alpha}$ in pulmonary circulation. Prostaglandins 16: 923-930, 1978.

Crutchley, D. J., Hawkins, H. J., Eling, T. E., and Anderson, M. W.: Covalent binding of prostaglandin PGG_2 and PGH_2 to discuss tissue macromolecules. Biochem. Pharm. 28: 1519-1523, 1979.

Wilson, A. G. E., Kung, A., Anderson, M. W., and Eling, T. E.: Covalent binding of arachidonic acid metabolites in platelets. Prostaglandin. In press.

Crutchley, D., Boy, J., and Eling, T. E.: Potentiation of TXB_2 release from guinea pig lung during anaphylaxis following exposure to 100% O_2. Submitted to Amer. Rev. of Resp. Dis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80035-03 LPFT

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Co-oxidation of Xenobiotics by the Prostaglandin and Thromboxane Synthetase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. E. Eling	Head, Prostaglandin Group	LPFT	NIEHS
Others:	K. Sivarajah	Visiting Fellow	LPFT	NIEHS
	J. C. Barrett	Senior Staff Fellow	LPFT	NIEHS

COOPERATING UNITS (if any)

Environmental Biology and Chemistry Branch
Duke University

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Prostaglandin Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.0	PROFESSIONAL: .9	OTHER: .1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long-range goal of this project to study the co-oxygenation of chemicals, i.e., benzo(a)pyrene (BP) 7,8-diol during the metabolism of arachidonic acid (AA) by guinea pig lung, ram seminal vesicle, mouse skin, microsomes, and cells grown in tissue culture. BP-7,8-diol was oxidized to 7,10/8,9 tetrol by prostaglandin synthetase by all the tissues examined. Electrophilic metabolites of BP-7,8 diol were formed since BP-7,8 diol was covalently bound to protein. Significance of this metabolic activation in relation to cell transformation is being examined.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Microsomal preparations of various tissues, such as guinea pig lung and ram seminal vesicles, were used to examine the co-oxygenation of xenobiotics during prostaglandin synthesis. The BP-7,8 diol metabolites were isolated from the incubation medium by extraction and by a preliminary separation by thin-layer chromatography techniques. Prostaglandin (PG) and thromboxane (Tx) products were also isolated and quantitated. Tissue cultures are used to examine the interaction of BP electrophilic metabolites produced by PG synthetase and to determine if this interaction is related to cell transformation. HPLC was used to isolate and characterize the metabolites formed.

MAJOR FINDINGS AND PROPOSED COURSE: The environmental carcinogen, BP-7,8-diol is oxidized during the conversion of arachidonic acid to PGs and TX. The lung, skin and seminal vesicle microsomes converted BP-7,8-diol to metabolites which migrated as a single peak on thin layer chromatography and corresponded to authentic samples of BP tetrols. The metabolites formed from BP-7,8-diol by the AA system, when examined by the HPLC, appeared to be 7,10/8,9 tetrols (major metabolite) with small amounts of 7,8, 9/10 tetrol. BP-7,8-diol if oxidized can generate 4 tetrols. The cytochrome P_{450} dependent system of oxidation of BP-7,8-diol could generate 4 tetrols that come from diol epoxide I and II (two tetrols from each). It appears that the two tetrols generated by the AA system came from diol epoxide I. The diol epoxides are the ultimate carcinogens of BP. Of the two diol expoxides, diol epoxide I is supposed to be more carcinogenic. However, we cannot conclusively say that those tetrols generated by the AA system came from diol expoxide I. It may be that tetrols were formed via a free radical. A recent report indicated the AA dependent metabolism of BP-7,8 diol generated highly mutagenic metabolites towards Salmonella typhimurium. The metabolites generated by the AA system from BP-7,8 diol BP by lung and ram seminal vesicle microsomes were the same in our HPLC analysis. BP-7,8-diol was oxidized by AA dependent system to metabolites which were electrophilic as indicated by BP-7,8-diol associated radioactivity remaining covalently linked to protein. From our previous work and present work, it appears the AA system of oxidation of BP-7,8-diol is different from that of cytochrome P_{450} system. It may be the co-oxidation of chemicals is dependent on the formation of hydroperoxide (PGG_2). Other hydroperoxides may support oxidation of chemicals. Hydroperoxide dependent cytochrome P_{450} oxidation of chemicals has been reported. Further evidence is required to show the nature or peroxidase which oxidized the BP-7,8-diol. In tissues like guinea pig lungs which contain both PGs and P_{450}, both enzymes may serve as a peroxidase. Studies are in progress to determine the relative importance of AA dependent oxidation. Our proposed course is as follows: 1) to study a number of environmental chemicals that can be activated by the AA system and to determine the nature of the electrophilic metabolites formed; 2) to study AA dependent oxidation in tissue cultures, to determine whether significant oxidation occurs in vivo and if so, whether the metabolites covalently bound to DNA and protein; and using tissue cultures and in vitro mutagenic test systems to assess the significance of AA dependent BP. Oxidation to cell transformation; 3) to examine if the P_{448} system and PG synthetase are associated, since it has been shown that compounds that are induced by P_{448} induce prostaglandin synthetase.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many xenobiotics are thought to exert their toxicity by means of reactive metabolites which are generated *in vivo* with very short half-lives. These reactive metabolites react with tissue macromolecules to produce toxicity such as carcinogenesis, mutagenesis, teratogenesis, etc. The prostaglandin synthetase system is found in most mammalian tissues and has particularly high levels of activity in the lung and kidney. Moreover, arachidonic acid can be released from its phospholipid storage sites by various types of stimulation, for example, irritation of lung tissue by inhaled pollutants. The subsequent metabolism of arachidonic acid by prostaglandin synthetase and the simultaneous co-oxygenation of xenobiotics could produce toxic metabolites of xenobiotics.

PUBLICATIONS

Sivarajah, K., Anderson, M. W., and Eling, T. E.: Metabolism of Benzo(a) pyrene to Reactive Intermediate(s) via Prostaglandin Biosynthesis, *Life Sci*. 23: 2571-2578 (1978).

Sivarajah, K., Mukhtar, H., and Eling. T. E.: Arachidonic acid dependent metabolism of ± trans-7,8-dihydroxy-7,8-dihydro benzo(a)pyrene to 7,10/8,9 tetrols. FEBS Letters. Submitted.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 25011 Z01 ES ~~25005~~-01 LPFT

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Prostaglandins in Tumor Promotion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. E. Eling	Head, Prostaglandin Group	LPFT	NIEHS
	J. C. Barrett	Senior Staff Fellow	LPFT	NIEHS
	E. E. Sisskin	Staff Fellow	LPFT	NIEHS
	B. E. Tainer	Biologist	LPFT	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Prostaglandin Group, Environmental Carcinogenesis Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.60	.35	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range goal of this project to determine whether tumor promotion by various agents, e.g., phorbol esters, proceeds via the release of arachidonic acid (AA) and the subsequent synthesis of prostaglandins (PG). We have examined several fibroblasts and chosen the mouse embryo fibroblast (10T 1/2) as a model system. PG production by these cells was examined with respect to cell growth, and cell density. The kind of PG produced by 10T 1/2 was dependent on the number of passages. Phorbal esters (TPA) altered cell growth and PG production. If a correlation can be determined between PG release and tumor promotion, we will have established a basis for the development of a test system for screening environmental tumor promoters.

PROJECT DESCRIPTION

METHODS EMPLOYED: Various fibroblasts were grown in culture under normal conditions. Mouse (10T 1/2), hamster embryo (HE) fibroblast, and a transformed derivative of HE (BP6T) were used in these studies. The cells were exposed to the tumor promoter 12-O-tetradecanoyil phorbol 13-acetate (TPA) for varying lengths time and at various concentrations. The culture growth media was then analyzed by specific radioimmunoassay antisera for PGs and thromboxanes.

MAJOR FINDINGS AND PROPOSED COURSE: The mouse embryo fibroblast (10T 1/2) was chosen as a model system for studying the correlation between prostaglandin (PG) formation from released arachidonic acid (AA) and tumor promotion by the phorbol esters (TPA). This fibroblast was chosen based on the high amounts of PG made by these cells and the ability of TPA to promote 10T 1/2 cell transformation. Production of PG by these cells was characterized; the amount of PG produced was highly dependent on cell density and growth.

Log growth cell produce high amounts of PG but at confluency (no growth) little or no PG were detectable in the media. 10T 1/2 was found to produce high amounts of 6-keto PGF_1, the decomposition product of PGI_2, and lesser amounts of PGE_2. Little or no $PGF_{2\alpha}$ or TXB_2 was detected. Exposing the 10T 1/2 cells to either a 24-hour treatment or a continuous treatment of TPA significantly altered the growth rate of the cells and the PG production. However, TPA treatment under these conditions did not appear to increase PG production via AA release but appeared to alter PG levels by altering growth rates. In a preliminary experiment, indomethacin, which inhibits formation of PG from AA, was only moderately successful in preventing TPA promotion after exposure to the carcinogen benzo(a)pyrene. The proposed course is (1) to further examine the effect of TPA or other promoters on the release of AA and the subsequent PG synthetase in 10T 1/2, (2) to use inhibitors of phosphalipase A_2 (steroids, meprine) in an attempt to differentiate the release of AA from phospholipids and the formation of PG released due to tumor promoters.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A number of non-carcinogenic chemicals increase the number of tumors observed and decreases the time before tumors appear after exposure to a carcinogen. The mechanism response for the promotion of tumors is not known. Recent reports have shown that a number of tumor promoters release arachidonic acid and stimulate PG production. This has been proposed as a mechanism of action for tumor promotion. At the present time, there is no simple adequate test to determine whether a chemical is or is not a promoter. If this hypothesis is correct then a test system for determining promotion potential of a chemical could be developed. Determination of the mechanism of action for tumor promoters would significantly add to our understanding of carcinogenesis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25006-02 LPFT

PERIOD COVERED
October 1, 1978 through September 30, 1979

TITLE OF PROJECT (80 characters or less)

Studies on Thromboxane Synthetase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. E. Eling	Head, Prostaglandin Group	LPFT	NIEHS
Others:	B. E. Tainer	Biologist	LPFT	NIEHS
	M. W. Anderson	Mathematician	LPK	NIEHS

COOPERATING UNITS (if any)

Laboratory of Pharmacokinetics

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Prostaglandin Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.0	PROFESSIONAL: .25	OTHER: .75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The unstable bioregulator thromboxane A_2 (TXA_2) is generated from the prostaglandin (PG) endoperoxide PGH_2 by an enzyme called thromboxane synthetase. Thromboxane synthetase is present in many tissues including blood platelets, lung, spleen and brain. Simultaneous with the formation of TXA_2 is the formation of C-17 hydroxy fatty acid (HHT) from PGH_2. We have investigated this process with emphasis on kinetics and inhibitors. We have shown that HHT (12-Hydroxy-5,8,11-hepadecenoic acid) is not a breakdown product of TXA_2 but that TXA_2 breaks down exclusively into thromboxane B_2. Moreover, our results indicate that thromboxane synthetase is not an isomerase but rather a bimolecular reaction is involved in the formation of TXA_2 and perhaps HHT. Also, we have preliminary evidence that there is another unstable intermediate formed from PGH_2 which breaks down to HHT. The existence of another intermediate poses interesting experimental questions concerning possible biochemical and biological activities.

PROJECT DESCRIPTION

METHODS EMPLOYED: Ram seminal vesicles microsomes were used to biosynthesize ^{14}C-PCH_2 or ^3H-PGH_2 from ^{14}C or ^3H-arachidonic acid. Washed human platelets and human platelet microsomes were prepared from platelets obtained from the Red Cross. The conversion of PGH_2 to TXB_2, (the stable endproduct of TXA_2) and HHT were measured using thin layer or high pressure liquid chromatography. Malondialdehyde formed during the production of HHT (12L-hydroxy-5,8,10-heptadecaenore acid) was estimated by a fluorescent thiobarbituric acid reaction. TXA_2 was trapped as the O-methyl derivative and measured on TLC or HPLC. The in situ formation of HHT was followed by measuring the absorbance of HHT at 236 nm using a DW-2 spectrophotometer.

MAJOR FINDINGS AND PROPOSED COURSE: Our data and reports in the literature show that TXB_2 and HHT are produced in approximately the same amounts when AA or PGH_2 are incubated with thromboxane synthesizing tissues. Inhibition of thromboxane synthetase appeared to inhibit the production of both TXB_2 and HHT to the same extent. One possible explanation of these observations is that TXA_2 could break down into either TXB_2 or HHT. However, our results explicitly show that HHT is not a breakdown product of TXA_2 but rather TXA_2 decomposes exclusively into TXB_2.

A model for the enzymatic generation of TXB_2 and HHT from PGH_2 has been proposed which is consistent with the above observation. It was suggested that PGH_2 can rearrange into either TXA_2 or HHT and malondialdehyde. Our enzyme kinetic results are consistent with this model. The rate of formation of TXB_2 is clearly dependent upon $[PGH_2]$ and thus a bimolecular reaction is involved in the formation of TXB_2. A reaction mechanism in which one molecule each of TXA_2 and HHT are produced simultaneously from two molecules of PGH_2 is consistent with our kinetic results as well as the above-mentioned observations.

We have been unsuccessful in our preliminary attempts to measure the rate of formation of HHT or MDA from PGH_2 to determine if a bimolecular reaction is involved in the formation of HHT. These studies have been complicated by the non-enzymatic break down of PGH_2 to HHT and the consequent difficulty of differentiating the enzymatic and nonenzymatic processes. In addition, these experiments require high specific activity PGH_2 which during purification decomposes nonenzymatically to HHT thus contaminating the substrate with the product we wish to measure. Alternately, we explored the use of a fluorescent assay for MDA to measure the rate of formation of MDA but this assay was not sufficiently sensitive. We were able to measure the formation of moderate concentrations of HHT by following its absorbance at 236 nm. The time course for the formation of a PGH_2 metabolite (in situ) absorbing at 236 nm, was significantly different from the HHT formation measured after extraction and analysis by TLC. Thromboxane synthetase inhibitor inhibits the in situ formation of 236 nm absorbing metabolite. These preliminary observations indicate that PGH_2 is converted to an unstable intermediate which breaks down during isolation to HHT. Thus, thromboxane synthetase appears to form two unstable intermediates, one of which (TXA_2) breaks down to TXB_2, the other to HHT. We plan to further investigate thromboxane synthetase and acquire additional evidence to support our hypothesis of an HHT intermediate. We propose to isolate or trap this unknown, characterize its biological activity and determine the mechanism for its formation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Thromboxane synthetase is present in many tissues including lung, blood platelets, spleen and brain. Thromboxanes have very potent effects on smooth muscle including that of lung airways. They have been implicated as primary mediators in platelet aggregation. A variety of stimuli can release significant amounts of thromboxanes from the lung. Since prostaglandins and thromboxanes are not stored in tissues, understanding the factors that control and regulate their biosynthesis is most important in understanding the physiological role of these bioactive substances.

The possible discovery of an additional unstable intermediate in prostaglandin biosynthesis with unknown biological activity may open new areas for the role of PG in biological processes.

PUBLICATIONS

Anderson, M. W., Crutchley, D. J., Tainer, B. E., and Eling, T. E.: Kinetic studies on the conversion of prostaglandin endoperoxide PGH_2 by thromboxane synthetase. Prostaglandins 16: 563-570, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES ~~25006~~ 25011-01 LPFT

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Lung as an Endocrine Organ Controlling Intravascular Thrombosis.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Ali	Visiting Fellow	LPFT	NIEHS
	T. Eling	Head, Prostaglandin Group	LPFT	NIEHS
	C. Barrett	Head, Environmental Carcinogenesis Group	LPFT	NIEHS
	J. Boyd	Technician	LPFT	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Prostaglandin

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	1.25	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this study is to unmask the role of pulmonary metabolism of essential fatty acids e.g. arachidonic acid in the etiology of intraarterial thrombosis. It is hoped that this study will determine (1) the factors controlling production of prostaglandins and thromboxanes by pulmonary tissue and lining vascular endothelium; (2) characterize the events which inhibit or promote platelet aggregation in vivo; (3) compare the response of pulmonary artery and pulmonary vein to stimuli which enhance the metabolism of fatty acids in vivo; (4) the mechanism(s) of action of chemicals and environmental agents (to be screened) which may stimulate intraarterial thrombosis; (5) the mode of secretion and transport of pulmonary prostaglandins and thromboxanes via capillary endothelium.

PROJECT DESCRIPTION

METHODS EMPLOYED: Prostaglandins and thromboxanes were measured biologically or by radioimmunoassay, thin-layer radiochromatography and high performance liquid chromatography with subsequent liquid scintillation counting. Platelet aggregability was measured by a "platelet-aggregation photospectometer." The metabolism of essential fatty acids as well as PG-transport studies were performed with monolayers of cells in culture or whole-cell homogenates incubated in vitro. Histological tools were used e.g. electron microscopy, immunohistochemistry, to identify and study metabolic activity of cultured cells (fibroblasts and vascular endothelial cells). The interaction between the pulmonary, vascular and human platelets was studied using the isolated perfused rat, guinea pig and rabbit lung.

MAJOR FINDINGS AND PROPOSED COURSE: A few cell lines other than those of endothelial origin can produce prostacyclin (the most potent anti-aggregatory prostaglandin that is biosynthesized by the endothelial cells). 10T 1/2 cells (a mouse embryo fibroblast line) SHE (hamster embryo fibroblast) are found to exemplify this category. Both cell lines produce also PGE_2 and $PGF_{2!}$ though in other cell types e.g. 3T3 PGE_2 is the major product whereas prostacyclin was unidentifiable. Cells from bovine aorta endothelium (ABAE) as well as bovine fetal heart endothelium (FBHE) were also tested for their ability to produce prostacyclin and it was confirmed that both these lines produce predominantly PGI_2 (prostacyclin) as the main product of arachidonic acid metabolism while they significantly lack the ability to produce thromboxane A_2 (monitored by measuring its stable metabolite TXB_2). On incubation of these lines (confluent monolayers with 20mM sodium arachidonate in Hepes buffer for 2 minutes at 37°C, the characteristic activity of prostacyclin as inibitor of platelet aggregation was monitored. Production of this activity could be prevented by incubation of the cells in media containing indomethacin or tranylcypromine (a PGI_2-synthetase inhibitor). However, a significant difference was observed between the different cell lines in that they produce varying amounts of prostacyclin-like activity depending on the tissue of origin. Thus ABAE cells were about 192% and 364% as active as FBHE and 10T 1/2 cells respectively as producer of PGI_2.

The cultured cells (in vitro) seem to produce more prostaglandins, be it PGE_2 or 6-keto $PGF_{1\alpha}$ (stable metabolite of PGI_2) during the logarithmic growth phase of the cell cycle. This tendency was abolished once the cultured cells reached confluency and this indicates a "shut-off" of arachidonate metabolism which paralleled postconfluence inhibition of proliferation and division. Also it was observed that indomethacin (a prostaglandin synthetase inhibitor that acts by cyclooxygenase inhibition) is more active against logarithmically growing cells compared to confluent monolayers as inhibitors if prostaglandin production.

Human platelets aggregated with ADP on passage through the vasculature of the isolated perfused guinea pig lung release very high quantities of TXA_2 and thus TXB_2 into the perfusate. Nonaggregated human platelets at perfusate concentrations similar to blood release little TXB_2 on passage through the vasculature. Increasing platelet concentration significantly elevates

TXB_2 levels detected in the perfusate. This data suggests that the PGI_2 produced by lung endothelial cells prevents aggregation or dissociates platelet aggregates reducing TXB_2 release. Origin of the TXB_2, either from lung or platelet is not known. The interactions between human platelets and guinea pig lung appears not to be the result of an immunological reaction since lactate dehydrogenase is not released during these experiments. Our proposed course is to (1) determine if platelets of lung produce the TXB_2; (2) add exogenous PGE_2 to the perfusate and examine if this PG will inhibit the release of TXB_2; (3) to further study the interaction of platelets with vascular endothelium of the lung employing antibodies which antagonize the effect of PGI_2 on platelets.

Having established proper conditions for isolation and culture of vascular endothelial cells from the cardiopulmonary region, it is now within our reach to study the factors which control the uptake and release of arachidonic acid by the aforementioned cells. These studies will utilize radiolabelled arachidonate and stimulates or blockers for phospholipase enzyme activity. Next, the question of the processes that control biosynthesis of PGI_2, which is derived from arachidonic acid, can be tackled in order to explain the relationship between the known mediators of intraarterial thrombosis (possible inhibitors of PGI_2 synthesis) and pulmonary vasculature.

It is envisioned that damage to the pulmonary vascular endothelium would activate formation of stationary or labile thrombi. Hence, a number of chemicals, drugs and environmental agents which are known to interfere with pulmonary function will be subject to a screening program to uncover their potential hazard as initiators or mediators of intravascular thrombosis. Moreover a study will be contemplated of the differences between the pulmonary arterial and venous circulations in response to agents which affect prostaglandin metabolism. Drugs that will be tested include thrombin, menadione, mepacrine, heparin, caffeine, troxerutin and bioflavanoids. Environmental agents to be screened are exemplified by cholesterol, nicotine, benzpyrenes, dimethylbenzanthracenes, 3-methyl-cholanthrene, butaylated hydroxytoluenes and phorbol esters.

To be studied in detail is the interaction of blood platelets (thrombocytes), which are a major source of pro-aggregatory thromboxanes and their endoperoxide precursors, with the pulmonary endothelium (i.e. lining capillary endothelium). This can be followed both in vitro with cultured cells or isolated perfused lung preparations, or in vivo with whole animal preparations. The guinea pig and/or the rabbit may serve as a satisfactory model in the latter case.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Cerebral and coronary strokes (heart attacks) as a result of intraarterial thrombosis constitute the major cause of death in this country. Little is known of the causes and the mechanisms that control the formation of intraarterial thrombosis. The lung with its vascular bed and extensive endothelial lining apparently play a major, yet unravelled, role in the control of platelet aggregation and thus thrombi formation. Changes in this endocrine function or pulmonary tissue by exposure to environmental agents may have an impact on the state of health of the exposed subjects to cerebral or heart strokes. We seek to understand the mechanisms that control the pulmonary formation of PGI_2 which should thereby significantly contribute to our understanding of and ability to prevent intraarterial thrombosis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80023-06 LPFT

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Composition and Origins of the Acellular Lining Layer of the Lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. E. R. Hook	Head, Biochemical Pathology Group	LPFT	NIEHS
	J. W. Spalding	Research Biologist	LPFT	NIEHS
Other:	D. Y. Bell	IPA	LPFT	NIEHS
	L. B. Gilmore	Biologist	LPFT	NIEHS

COOPERATING UNITS (if any)

Environmental Chemistry and Biology Branch

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Biochemical Pathology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The alveoli and distal airways of the lung are lined with an acellular layer of material which is essential for the maintenance of normal pulmonary functions such as gas exchange. The composition and origins of the acellular lining are being investigated. Current attention has been directed towards: (1) the biosynthesis and secretion of pulmonary surfactant and (2) the origins of several extracellular enzymes present in the lining.

The objectives of this investigation are as follows: (1) to elucidate the composition of the acellular lining; (2) to identify processes by which these components arise in the acellular lining; (3) to identify the subcellular processes involved in the formation of acellular lining components synthesized by the lungs; (4) to identify variations in the acellular lining of diseased and damaged lungs.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Acellular lining material is obtained by lavaging the lungs of rabbits via the trachea. Lamellar bodies as found in the alveolar type 2 cells are isolated using differential centrifugation on discontinuous sucrose gradients. Enzyme and protein analyses are carried out using polyacrylamide gel electrophoresis.

MAJOR FINDINGS AND PROPOSED COURSE: Previous studies from this laboratory have shown the presence of several highly active hydrolytic enzymes in the acellular lining of the pulmonary airways and alveoli. The hydrolases of the acellular lining appear to be secreted by the alveolar type 2 cells during their secretion of lamellar bodies. The hydrolases which are normally considered to be of lysosomal origins appear to be components of the lamellar bodies. We have investigated the hydrolase content of lysosome-free lamellar bodies and compared them with lamellar body-free lysosomes. Of 23 hydrolases studied in the pulmonary lysosomes, all were present in the lamellar bodies including phospholipase A. The presence of phospholipase A in lamellar bodies indicates that since the major component of the lamellar bodies is phospholipid, constraints must be placed upon the hydrolase to prevent dissolution of the structure. These data indicate that lamellar bodies may be derived from lysosomes. Future studies will involve the lamellar body compartments in which the hydrolases are confined. Preliminary evidence using chaotropic agents such as detergents and high concentrations of sodium chloride, indicates that acid phosphatase and β-N-acetylglucosaminidase are localized in different regions of the lamellar body.

Biochemical processes involved in the formation of lamellar bodies have been investigated. Phospholipid transfer proteins present in the pulmonary cytosol were shown to facilitate the transfer of phosphatidylcholine and phosphatidylinositol from microsomes, mitochondria, and synthetic liposomes, to isolated lamellar bodies. The transfer rate was proportional to the amount of transfer protein present and was linear with time up to 30 mins. The studies also required the development of a method for the isolation of lamellar bodies in a highly pure and structurally intact form. Morphologically, under the electron microscope the isolated lamellar bodies were in excellent condition and in a highly purified form. Studies of the lamellar bodies will continue to involve mechanisms through which these structures are formed. Attempts will be made to force lamellar bodies to incorporate phospholipid into their structure using radiolabelled phospholipids and phospholipid precursors in the presence of cytosol and cofactors such as ATP.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The acellular lining of the lung is vital for the maintenance of normal lung functions such as gas exchange. Inhaled toxicants such as the oxidant gases (e.g., ozone), particulate materials (e.g., silica) and chemicals (e.g. paraquat appear to affect the acellular lining both qualitatively and quantitatively. The involvement of the acellular lining in the progression and mediation of some pulmonary diseases such as alveolar proteinosis appears certain. Unfortunately, the mechanisms which underlie these pulmonary diseases and agent-induced lung damage are not known. Elucidation of the biochemical processes which contribute to the formation of pulmonary surfactant and the acellular lining are a necessary step in the understanding of the disease process. For example, an absence of pulmonary surfactant in diseases such as the respiratory distress syndrome of the newborn could be due to malfunctions in the transport of phospholipids about the type 2 cell or to an inability of the cell to form lamellar bodies.

PUBLICATIONS

Reasor, M. J., Nadeau, D., and Hook, G. E. R.: Extracellular alkaline phosphatases in the airways of the rabbit lung. Lung 155: 321-335 (1978).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80029-03 LPFT

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Investigations of Human Pulmonary Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. E. R. Hook	Head, Biochemical Pathology Group	LPFT	NIEHS
	D. Y. Bell	IPA	LPFT	NIEHS
Other:	S. E. Fabro	Consultant	LET	NIEHS

COOPERATING UNITS (if any)
Department of Medicine, Duke University; Environmental Biology and Chemistry Branch; Department of Gynecology and Obstetrics, George Washington University

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Biochemical Pathology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.2	PROFESSIONAL: 1.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with the identification and characterization of components of the lung associated with pulmonary diseases which could provide information concerning the disease processes as well as function as diagnostic markers of pulmonary damage or disease.

Current attention has been focused on the protein components of the acellular lining of the normal human lung and the lungs of patients with pulmonary alveolar proteinosis. The objectives are to identify and ennumerate the protein constituents in the acellular lining of the normal lung and thus provide a baseline against which disease and lung damage may be measured.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Lavage effluents from normal human lungs were obtained from the University of Iowa Medical Center. These lavage effluents were obtained using the fiberoptic bronchoscope from the lungs of healthy human volunteers.

Bronchoalveolar lavage effluents from patients with the pulmonary disease alveolar proteinosis were obtained from the Department of Medicine at Duke University. These lavage effluents were obtained as a by-product of the therapy essential to the well being of the patients.

Insoluble materials were removed from the lavage effluents by differential centrifugation. The lavage effluents were concentrated using an ultrafiltration apparatus. Proteins were resolved using two-dimensional polyacrylamide gel electrophoresis in which the proteins were first separated according to their electrophoretic mobilities in 4% polyacrylamide gel rods and then separated according to their molecular size in linear gradient (2.5-27% acrylamide) polyacrylamide gel slabs. The protein maps thus obtained for lavage effluent proteins were compared with proteins from the volunteer's or patient's serum.

Plasma proteins were measured quantitatively using radial immunodiffusion.

MAJOR FINDINGS AND PROPOSED COURSE: Twenty-three plasma proteins were measured in six samples of normal human lung lavage effluents and matched sera, by radial immunodiffusion. All samples, obtained by segmental lavage with a fiberoptic bronchoscope, were from healthy non-smoking adults between 22 and 40 years of age. Seventy-six percent of the total lavage effluent protein and 99% of the serum protein could be accounted for. Of the five immunoglobulin classes, only IgG and IgA were detected in lavage effluents: IgG (9.7% of the total protein) was more abundant than IgA (2.4% of the total protein). Sera had normal immunoglobulin levels for this age group. Non-immunoglobulin proteins of low to intermediate molecular weight (<150,000 daltons) were present in lavage effluents in proportions similar to that found in serum. Proteins with higher molecular weights such as ceruloplasmin (150,000) the C_2 components of complement (185,000), and α_2-macroglobulin (820,000) were markedly reduced in lavage effluents being present at only 27, 21 and 1.4% of their serum levels, respectively. The β_1-lipoprotein (3,200,000) of serum was not detected in lavage effluents. The data indicate that most of the proteins in the EL are of plasma origin. However, not all plasma proteins are present, and those with molecular weights above 150,000 daltons tend to be excluded.

In lavage effluents from patients with pulmonary alveolar proteinosis, nineteen proteins were quantitated by radial immunodiffusion against antisera monospecific for normal plasma components. Most soluble phase proteins in lavage effluents from patients were present in their sera, although some species were absent (β-1-lipoprotein) or present in much lower proportions (haptoglobins, α-2-macroglobulin) than in patient serum.

Two proteins with molecular weights of 47,000 and 52,000 daltons were found in lavage effluents from patients which were present in sera or in lavage effluents from non-diseased human lungs or from the lungs of patients with cystic fibrosis or bronchial asthma. These proteins may be unique to pulmonary alveolar proteinosis and consequently of diagnostic value.

Uniformly elevated levels of immunoglobulins (400% of normal) found in patient lavage effluents and the presence of abnormal immunoglobulin levels and C-reactive protein in patient sera suggests involvement of an atypical hypersensitivity reaction.

This work will be expanded to include lavage effluents from the lungs of smokers and patients with other lung diseases such as cystic fibrosis and bronchial asthma. Unusual proteins will be sought and the presence or absence of proteins with molecular weights above 150,000 daltons used to assess blood/air permeability.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: 1) Many human pulmonary diseases have been described but unfortunately the diagnosis of these diseases is not usually made until the disease is well advanced. X-ray methods generally are not capable of detecting pulmonary diseases excepting in the advanced states and even then are often incapable of distinguishing between diseases. Methods for the diagnosis of pulmonary disease and the detection of pulmonary damage in the earliest possible stages are needed most urgently.

Our studies of the protein constituents of the acellular lining of the normal human lung have established a baseline against which diseased and damaged lungs can be measured. For example, the molecular weights of the proteins present and their concentrations indicate that the blood/air barrier in the lungs of patients with alveolar proteinosis is not markedly damaged and that the disease-associated elevation of soluble protein (20-40 grams) present in the acellular lining originates from the plasma through channels of approximately normal size and selectivity. In addition, the two proteins present in lavage effluents from patients but not in normal humans may eventually prove useful in diagnosis and monitoring of alveolar proteinosis.

PUBLICATIONS

Hook, G. E. R., Bell, D. Y., Gilmore, L. B., Nadeau, D., Reasor, M. J., and Talley, F. A. : Composition of bronchoalveolar lavage effluents from patients with pulmonary alveolar proteinosis. Lab. Invest. 39: 342-357 (1978).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25007-01 LPFT

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Particle Translocation in Various Cell Types and Anatomic Regions of the Lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. R. Brody	Senior Staff Fellow	LPFT	NIEHS
Other:	L. Hill	Chemist	LPFT	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Pulmonary Pathology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 3.0	PROFESSIONAL: 1.0	OTHER: 2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is known that small (0.5-20.0μ) inhaled inorganic particles normally reach the alveolar ducts and air spaces. There the particles confront various components of the alveolar wall, such as surfactant, epithelial cells, and alveolar macrophages. The initial events of particle interaction with these structures are poorly understood and relate directly to how certain particulates are distributed in the lung and exert their toxic influences. We have found that after one hr. inhaled chrysotile asbestos is found exclusively in the small air spaces. After one day of exposure, fibers are found in alveolar macrophages, and after five days asbestos reaches the alveolar epithelium, interstitium, and capillary spaces. Apparently, inhaled asbestos reaches the connective tissue compartment of the lung by inter- or intraepithelial particle transport.

PROJECT DESCRIPTION

METHODS EMPLOYED: Rats were exposed in inhalation chambers to chrysotile asbestos fibers at a concentration of 10 mg/m^3. After exposure, the animals' lungs were fixed in situ by infusion into the trachea of 2% glutaraldehyde at 20 cm of H_2O pressure. Tissue was prepared for light, transmission, and scanning electron microscopy by conventional techniques.

MAJOR FINDINGS AND PROPOSED COURSE: Following one hour of exposure and immediate sacrifice, numerous fibers were located in the small air spaces of the lung parenchyma, but none were found in any tissue compartment. After 7 hours of exposure and 16 hours recovery in room air, fibers were found in the alveolar air spaces and within alveolar macrophages. Macrophages were more numerous than in controls and often contained several clusters of fibers most of which were membrane-bound. Animals exposed for 7 hours on each of 5 successive days were sacrificed on the 6th day. At this time, fibers were located in numerous alveolar macrophages, within alveolar type I cells, within type II cells and between these epithelial cells. In addition, fibers were frequently found in subepithelial basement membranes and also were located within interstitial cells.

Studies necessary to more precisely define the intrapulmonary movements of inhaled asbestos currently are being designed. The ideal situation for such investigations is one where a short term (1-3 hr.) pulse dose of intermediate range asbestos is given to a large group of animals. Then at selected time periods (e.g. 0, 3 min., 1 hr., 4 hrs., 8 hrs., and daily for 7 days) groups of animals can be sacrificed and studied by light and electron microscopic techniques to quantitate numbers of particles in various anatomic compartments and then to determine the nature and degree of cytotoxic alterations which may occur. Such studies will be initiated in the Fall of 1979.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Pulmonary disease caused by exposure to inhaled inorganic dust usually is expressed as an inflammatory, fibrotic, or emphysematous response. The pathogenetic events of these processes are not well understood. Of particular interest are silica, asbestos, talc, and kaolinite, i.e., toxic particulates to which individuals commonly are exposed.

Finding chrysotile asbestos in the air spaces after 1 hour, in alveolar macrophages after 1 day and within the epithelium, basement membrane and interstitium after 5 days suggests that inhaled fibers reach the connective tissue compartment of the lung by intra- or interepithelial particle transport. This could be a highly significant mechanism for the introduction of small asbestos fibers to the interstitial lymphatic network and for the expression of cytotoxic events which precede asbestosis and neoplasia. We believe that elucidation of initial events and cell-particle interactions in the lung after inhalation of a toxic dust will lead us toward a better understanding of the basic mechanisms of dust-related pulmonary disease.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25008-01 LPFT

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Elemental Analysis of Asbestiform Minerals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. R. Brody	Senior Staff Fellow	LPFT	NIEHS
Other:	L. Hill	Chemist	LPFT	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Pulmonary Pathology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 3.0	PROFESSIONAL: 1.0	OTHER: 2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Chrysotile asbestos is a fibrous silicate used commonly in a number of manufacturing processes. This serpentine mineral is composed primarily of magnesium (Mg) hydroxide and silicon (Si) dioxide ($Mg_6(OH)_8Si_4O_{10}$). Magnesium ions are readily translocated from chrysotile fibers in a variety of media (e.g. saline at body temperature, organic and inorganic acids) and in human lungs. The toxicity of magnesium-leached fibers seems to be altered under certain experimental conditions. We are investigating in vitro and in vivo systems in which the elemental content of chrysotile asbestos can be critically evaluated and correlations with cytotoxic events can be made.

Treatment of aliquots of chrysotile asbestos with NHCL causes a slow translocation of Mg ions so that the ratio of Mg to Si changes from a control level of .71 to .49 after 8 hrs. Such changes do not occur after aldehyde fixation or plastic and paraffin embedding. Inhaled chrysotile asbestos in the lungs of rats showed clear changes in elemental ratio.

PROJECT DESCRIPTION

METHODS EMPLOYED: Whether or not elemental changes occur in chrysotile asbestos after treatment with commonly used reagents is an open question. Therefore, 0.25 gm samples of asbestos were treated with 2.5% glutaraldehyde, 2% Osmium tetroxide, 10% formalin and 1N HCl. Additional samples were dehydrated in 100% ethyl alcohol, propylele oxide, and xylene, then they were embedded in paraffin or plastic and sectioned for light and electron microscopy. In addition, lungs of asbestos-exposed rats were prepared for light and electron microscopy.

Two electron beam instruments were used to collect elemental data from the control asbestos samples and asbestos in the rat lung tissue, a scanning electron microscope (SEM) (JEOL, JSM-35C) and a transmission electron microscope (TEM) (Philips 300). Attached to both microscopes are x-ray energy spectrometers (XES). XES allows for the rapid and precise determination of elements from sodium through uranium. This is accomplished by focusing the electron beam on a region of interest during routine image formation in the SEM and TEM. The primary electrons which provide the image collide with and displace orbiting electrons in the atomic framework of the specimen causing the release of x-rays as the electrons return to their original orbits. These x-rays are collected from the microscope column through an XES detector and their specific energies are sorted out visually into specific elemental windows. In addition to the visual spectrum, x-ray counts (numbers of x-rays detected per unit time) are collected for elements of choice. These counts provide the numerical data used to calculate elemental ratios (see below).

Mg and Si are the major elemental components of chrysotile asbestos. Accordingly, x-rays collected in 100 sec. counting time were recorded by computer for the two elements. A ratio then was calculated simply through dividing the number of counts achieved for Mg by the counts for Si. Non-specific x-ray counts (bremstrahlung background) were automatically subtracted through computer programming.

MAJOR FINDINGS AND PROPOSED COURSE: To demonstrate the capability of our analytical system for detecting changes in elemental ratios, a control Mg to Si ratio was established for untreated asbestos. This value is .71 with a standard deviation of .04 determined from over 150 analyses on 36 fibers of varying lengths and diameters. Treatment of fibers with 1N HCl at room temperature causes a gradual loss of magnesium ions so that after 8 hrs., the Mg/Si ratio is .49 ± .04 (N = 20 fibers). Aldehyde fixation, dehydration and embedding did not significantly alter the elemental ratios.

Inhaled fibers in the lungs of rats exposed to asbestos for 3 months showed significant alterations in Mg/Si ratios. Particles in the small airspaces of the parenchyma had a mean elemental ratio of .30 ± .03 (N = 48). In contrast, particles and fibers distributed in interstitial connective tissue and cells had a mean Mg/Si ratio of .58 ± .09 (N = 25).

Thus, we have determined the elemental ratios of Mg/Si in individual chrysotile asbestos fibers after treatment with acids, fixatives, dehydrating agents and embedments. In addition, we have shown that changes in elemental ratios of chrysotile fibers can be detected *in situ* in the lungs of rats after inhalation. We do not know by what mechanisms or biological components the fibers are changed. The experiments to elucidate the nature of this phenomenon in animal lungs and in a variety of pulmonary cells are in the design stage and will be initiated shortly.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It has been suggested that magnesium leaching exposes more hydrogen donating silica complexes which may combine with surrounding cell and lysosomal membranes resulting in cytotoxic events. This mechanism may be operative in the lung subsequent to asbestos inhalation and could play an important role in the pathogenesis of fibrotic lung disease. Indeed, products associated with the death of alveolar macrophages stimulate the proliferation of fibroblasts and connective tissue *in vitro*. This is likely to be an important mechanism in dust-related pulmonary disease, and we plan to correlate cytotoxic events *in vitro* and *in vivo* with carefully defined changes in the elemental content of asbestos.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25009-01 LPFT

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Structure and Function of the Clara Cells of Lower Airways

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. G. Plopper	Senior Staff Fellow	LPFT	NIEHS
Other:	R. Philpot	Biochemist	LP	NIEHS
	L. H. Hill	Chemist	LPFT	NIEHS
	C. Schull	Chemist	LP	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Pulmonary Pathology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.5	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Three physiologic roles in normal lung function have been proposed for the non-ciliated epithelial (Clara) cells lining small conducting pulmonary airways (bronchioles): 1) secretion of airway lining material; 2) metabolism of chemical toxins and carcinogens; and 3) progenitor cell for bronchiolar epithelium. The goal of this line of investigation is to clarify these separate functions of the Clara cell with emphasis on its role in detoxification and carcinogen metabolism, and on the interrelationship between these various functions. Work is progressing along the following lines. First, a comparative study of the cellular characteristics of this cell type in the lungs of twelve species of mammals, including man, has been undertaken to define the Clara cell as a lung cell type and to identify species that could serve as useful models for subsequent investigations. Second, the detoxification function will be characterized in terms of the cellular response during exposure, and the time course of events during the response and subsequent recovery phase. Third, the actual site of the cytochrome P-450's among the various lung cell types and their intracellular distribution will be determined using the unlabelled antibody immunoperoxidase technique.

PROJECT DESCRIPTION

METHODS EMPLOYED: This project has been used to develop techniques for the embedding of large blocks of lung tissue for sequential light and transmission electron microscopy, and to train a technician in their use. These techniques have allowed us to specifically identify the anatomic location of airways from which Clara cells were characterized. All lungs studied were fixed and embedded by a standardized procedure. Thus far, we have examined airways from lungs of the following species (no. of individuals): man(3), horse(3), cattle(12), sheep(12), dog(3), cat(3), rabbit(4), guinea pig (4), rat(6), Syrian hamster(8), mouse(3). Only cells lining the most distal non-alveolarized airway, in which the plane of section included the base, apex and nucleus of the cell, are to be described.

In order to identify pulmonary cell types contianing cytochrome P450, we will use specific antisera and purified antibodies made against the two purified forms of the cytochrome to localize the cytochrome in lung tissue. The cytochromes were isolated from rabbit lung. The unlabelled antibody immunoperoxidase technique will be used (Sternberger, et. a., J. Histochem. Cytochem., 1970). First, paraffin sections of unstimulated rabbit lung will be reacted to identify by light microscopy cell types containing the antigen and to evaluate optimum parameters for lung fixation and processing. Then, the technique will be modified for ultrastructural localization of antigen within cells. Finally, cells of lungs from rabbits exposed to ipomeanol will be used for localization studies.

MAJOR FINDINGS AND PROPOSED COURSE: Nonciliated bronchiolar epithelium of the horse, sheep, rabbit, guinea pig, rat, hamster, and mouse contained electron-dense membrane bound apical granules and abundant agranular endoplasmic reticulum. Nonciliated bronchiolar epithelium of cattle and cat was nearly devoid of granules and agranular endoplasmic reticulum. The cytoplasm was filled with glycogen. Nonciliated bronchiolar epithelium of the dog exhibited ovoid granules, agranular endoplasmic reticulum and a great abundance of cytoplasmic glycogen. The Clara cell of man contained little agranular endoplasmic reticulum and moderate numbers of secretory granules and granular endoplasmic reticulum. In all species except dog and cat, the non-ciliated cells were between 50 and 75% of the bronchiolar epithelial population. In the dog and cat, the Clara was almost 100% of the bronchiolar epithelial population in the most distal airway and about 90% in the next proximal bronchiole.

Our observations thus far indicate that there is considerable variation in the cellular morphology of the nonciliated bronchiolar epithelial cell among mammalian species. If the nonciliated cell of distal airways is to be considered a unified cell type, then it appears that there are at least three categories of this cell type: 1) a cell with an abundance of secretory granules and agranular endoplasmic reticulum, presumably actively involved in both secretory and detoxification functions, which is present in laboratory and some domestic animals; 2) a cell with moderately abundant secretory granules and no agranular endoplasmic reticulum, presumably an active secretory cell without detoxifying capabilities, which is present in primates; and 3) a cell with minimal secretory granules and agranular endoplasmic reticulum but large amounts of glycogen, presumably with little secretory or detoxification capability, which is present in cattle, dogs and cats. Work by others indicates that this is true for pigs as well.

Two series of studies are planned or have been initiated. One series will aim to: 1) characterize the cellular reaction to exposure to a pulmonary chemical toxin, including early reaction and long term response, in the airway epithelium of a species which has nonciliated cells with large amounts of agranular endoplasmic reticulum; 2) ascertain the reaction of non-ciliated cells to toxins in the lung of a species where agranular endoplasmic reticulum is not a major cytoplasm component, such as the cat. The other will: 1) identify pulmonary cells types containing cytochrome P450; 2) localize the cytochromes to specific cytoplasmic organelles.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The pulmonary system is the most vulnerable organ system in the body to damage from atmospheric pollutants. Understanding which of the forty some odd cell types composing this system are most susceptible, how they react, and which ones function in a defensive role, is vital to an understanding of how mammals react to toxicants. The nonciliated bronchiolar epithelial (Clara) cell is one of the least understood of the cell types within the system. Many roles in lung function have been proposed: secretion, detoxification, and cell renewal, but none of these roles has been clearly defined, nor has the relationship between them been elucidated. This project will attempt to resolve these questions.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25010-01 LPFT

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of Perinatal Exposure to Gaseous Pollutants on Lung Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. G. Plopper	Senior Staff Fellow	LPFT	NIEHS
Other:	L. H. Hill	Chemist	LPFT	NIEHS

COOPERATING UNITS (if any)

California Primate Research Center, University of California, Davis

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Pulmonary Pathology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

While the pulmonary damage resulting from acute, short-term exposure to components of photochemical smog have been well characterized biochemically, morphologically and physiologically in adults, very little information is available concerning the mechanisms by which the respiratory system adapts to continual insult over the lifetime of an organism. The goal of this work area will be to elucidate the nature of the interactions between the cells of the growing lung and environmental irritants. The emphasis will be on pulmonary reactions occurring over the entire life span of the organism (prenatal to death) in response to gases at realistic environmental concentrations. The first phase is to characterize the normal cellular events during prenatal and early postnatal development of the mammalian lung of a non-human primate (*M. mulatta*). Once the usefulness of the rhesus monkey as a model for studies of pollutant effects on lung development, specific protocols will be developed to evaluate pollutant effects on alveolarization, cellular maturation and overall lung growth and repair phenomena.

PROJECT DESCRIPTION

METHODS EMPLOYED: A collection of lungs from fetal and postnatal rhesus monkeys has been made available for this study by L. W. Schwartz of the California Primate Research Center.

In all cases, the lower third of the trachea, right bronchus and right upper and middle lobes will be used. All were fixed by airway infusion at constant pressure. Fixed lung volumes were measured by fluid displacement for lungs which were still intact at the time the study began. Methods are now under development to extend the techniques used for study of the Clara cell to enable identification of airways by generation of branching (trachea is considered generation #0) and scanning and transmission electron microscopy of complementary halves of airways of known generation. This selective large block embedding technique will be used to embed entire halves of trachea, major bronchi and parenchyma for transmission e.m. Complimentary halves will be examined by scanning e.m. The combination of techniques allows specific localization of areas for high resolution examination and very close correlation between cellular surface characteristics observed by scanning e.m. and internal cellular morphology observed by transmission e.m.

MAJOR FINDINGS AND PROPOSED COURSE: Thus far, microdissection techniques and processing of complementary halves have been applied to three sets of lungs from 91-, 120-, and 164-day-old animals. Good correlation appears to be possible between scanning and transmission e.m. Careful record keeping during dissection followed by macrophotography of specimens has proven essential for accurate identification of airway generations. We have successfully evaluated upper airways out to the sixth generation in the upper right lobe. We are now proceeding to dissect and process the rest of the collection.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Over forty cell types have been identified in the lower respiratory system of adult mammals. The origin, time of appearance and sequence of differentiation of only two of the fifteen types of epithelium has been clearly established. A similar situation exists for the twenty-four cell types of mesenchymal origin. The basic transformation of mesenchymal cells has been established and some of the phases of secretion characterized. The interdependence of entodermal and mesenchymal derivatives in normal fetal lung development is now becoming apparent. Pulmonary embryogenesis occurs only in the presence of specific mesoderm, and intimate contacts, through the basal lamina, between epithelium and mesenchymal cells have been observed at the ends of growing lung buds in embryonic mouse lung. Whether this occurs in other species and for how long during development has not been ascertained. Since epithelial-mesenchymal contact across the basal lamina has now been reported in alveoli of diseased adult human lung, it appears that a great deal has yet to be learned about the factors controlling pulmonary epithelial growth and differentiation during both lung maturation and lung repair and the relationship of mesenchymal cells to these processes. While the phases of architectural change during development of non-human primate lung have been well demonstrated (Boyden, Anat. Rec., 1976), virtually no data is available on cellular changes in trachea, bronchi, or lungs of a non-human primate species. The availability of a collection of fetal lungs from a species of a nonhuman primate offers a unique opportunity to study all aspects of cellular development in a species closely related to man. Since a considerable amount of data is already available on man, thorough assessment of the utility of the rhesus monkey as a model for human lung development will be possible. In addition, this study should provide information relevant to the development of epithelial and mesenchymal derivatives in the trachea, major bronchi, and bronchioles and correlation of cellular events at various levels of the respiratory tree during development.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25004-02 LPFT

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Establishing Pulmonary Epithelial Cells in Culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Reen Wu	IPA	LPFT	NIEHS
Others:	D. Smith	SIS	LPFT	NIEHS
	T. Devereux	Research Biologist	LP	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pulmonary Function and Toxicology

SECTION

Pulmonary Cell Biology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.0	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our research efforts are directed toward investigating the life cycle and basic functions of each type of pulmonary epithelial cells in a well-defined in vitro situation. A major objective of the research is to develop conditions for the long-term cultivation of normal, differentiated epithelial cells. Such a culture offers a model system to investigate the mechanism of cell differentiation, as well as the repair of injury and the pathological response of cells to environmental toxic substances. The success in culturing normal epithelial cells from the respiratory tract is very limited, since most epithelial cells in the conventional culture condition are short lived and cellular basic functions are rapidly lost. Our approach is on one hand to eliminate those unnecessary factors from the conventional culture condition and on the other hand, to provide only required elements for cells. The first study of these series is to replace serum by the needed hormones and growth factors. Our results indicate that tracheal epithelial cells can be continuously maintained in a hormone-supplemented medium. These hormones and factors needed for tracheal cell cultures are insulin, epidermal growth factor and transferrin.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Tracheal epithelia were exposed to 0.5 to 1.0% pronase solution at 37°C for 1 hour. Tracheas were then flushed with ice cold MEM medium containing 10% fetal calf serum. Epithelial cells were isolated from the flush fluid by centrifugation. Cells were resuspended in the F12 K medium and plated into various culture conditions.

MAJOR FINDINGS AND PROPOSED COURSE: Isolated rabbit tracheal epithelial cells placed in serum-containing culture condition, initially form small compact colonies of polygonal cells with smooth boundary. In this early stage of culture, cells shown a condensed cytoplasm are small and have a maximum cell to cell contact. After 2 to 3 days in culture, the cells become enlarged and the cytoplasm gradually loses its condensed feature. A week later some cells slough off from the dish, while some remain attached. Beating cilia are observed up to 4 days in culture. The addition of insulin (5-10 μg/ml) and hydrocortisone (0.1-1.0 μg/ml) help to preserve the culture longer (maximum 2 weeks). The addition of other hormones and factors (fibroblast growth factor, thyrotrophin releasing factor, luteinizing hormone, thyroxine, parathyroid hormone, calcitonin, prolactin, growth hormone, estrogen, testosterone, etc.) into the 5% serum-containing medium does not prevent the deterioration of the tracheal cell cultures. However, when the serum concentration in the media was lowered to 0.3%, a hormone effect became evident. The hormones and factors having a positive effect were: insulin, transferrin and epidermal growth factor. A combination of these factors in 0.3% serum medium showed a synergistic effect. The observed effects include an increase in cell number and a change in the morphology. The tracheal cells grew as a continuous sheet, and most cells were small and compact with condensed cytoplasmic features and granules. Under these conditions, the primary cultures can be maintained up to 2 months without passage. Cells can be passaged for three times by trypsin treatment. Preliminary studies indicated that these cells grown in the hormone-supplemented medium were stained positively with a PAS stain and with anti-keratin antibody. These results suggested that these cells are epithelial origin and some basic functions are preserved in the hormone-supplemented medium.

We have shown here that a drastic improvement in the maintenance of primary cultures of tracheal epithelial cells by reducing the serum contant in the medium. A similar approach can be applied to culture other types of pulmonary epithelial cells, such as, alveolar type II cells, Clara cells and APUD cells. It is our goal to establish a complete serum-free hormone-supplemented medium for cells.

The origins and cell basic functions of these epithelial cells in culture remain to be established. We would like to demonstrate the presence of or to induce differentiated functions, such as mucin synthesis, drug-metabolizing activity and the formation of cilia.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Within the last decade, culture techniques have been developed that permit differentiated cells derived from normal tissues to retain specialized characteristics while undergoing a series of replicative cycles. The advantage of studying a specific cell type in culture is apparent: the number of variables to be controlled is markedly reduced. With more than 40 different types of cells having been identified in the lung, the importance to study these cells *in vitro* is quite apparent.

We have shown here that the conventional usage of serum in culture is the major obstacle to the long-term culture of tracheal epithelial cells. A simple way to interpret the result is to say that serum contains unnecessary factors which have adverse effects to cells in culture. In order to culture cells continuously, it is necessary to remove serum from the medium and supplement only the essential hormones and growth factors for cells. The approach reiterates the *in vivo* situation that cells are regulated by hormones.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25000-02 LPFT

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Cultured Epidermal Cells as a Model for Skin Carcinogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. E. Sisskin	Staff Fellow	LPFT	NIEHS
	J. C. Barrett	Senior Staff Fellow	LPFT	NIEHS
Other:	S. H. Yuspa	Section Head	EPB	NCI

COOPERATING UNITS (if any)

Experimental Pathology Branch, NCI

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Environmental Carcinogenesis

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.25	PROFESSIONAL: 1.25	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The vast majority of human neoplasms is thought to be due to environmental factors. There is overwhelming evidence that skin cancer, one of the most commonly occurring human cancers, is usually due to exogeneous insult. The cells that are most likely to be progenitor cells for this carcinoma are the basal cells, which are rapidly dividing and/or differentiating, and as such make an excellent target cell for carcinogenic agents. The purpose of this work is (1) to develop an in vitro system consisting of dividing, basal cells isolated from epidermis, (2) to induce these cells to undergo neoplastic transformation in vitro following treatment with environmental carcinogens and/or promoters, (3) to determine whether the two-stage model of carcinogenesis is a universal phenomenon, common to animals other than the mouse, (4) to isolate cells from skin papillomas and carcinomas induced in vivo, and (5) to compare the cellular properties of epidermal cells at various stages after in vitro treatment with carcinogens to cells derived from papillomas and carcinomas. The work will be done with inbred Syrian hamsters.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Epidermal cells are isolated from newborn Syrian hamsters (Lakeview or Telaco strain 87.20 or 15.16) by surgically removing the skin from the animals, floating the skin (dermis side down) on 0.25% trypsin for 20 hrs. at 4°C, physically pulling the epidermis from the dermis and dissociating the epidermal cells by physical disruption. The epidermal cells are then filtered to a single cell suspension and plated in various media.

In order to measure the degree of differentiation, the number of cells with cornified envelopes is determined as a percentage of the total cell number. To do this, the cells are trypsinized and resuspended in buffer containing SDS and β-Mercaptoethanol. This solution lyses all the cells except the ones with cornified envelopes which can then be counted.

For tranformation studies, the cells are treated at different times with carcinogens such as benzo(a)pyrene, N-methyl-N-nitro-N-nitrosoguanidine, dimethylbenzanthracene or 3-methylcholanthrene and promoters such as phorbol esters.

In vivo tumor promotion studies with hamsters are also in progress. Inbred Syrian hamsters are initiated with B(a)P or DMBA and promoted with phorbol ester derivatives.

MAJOR FINDINGS AND PROPOSED COURSE: Epidermal cell cultures from newborn inbred and outbred Syrian hamsters have been established. The epithelial nature of these cultures was determined by morphological criteria at the light microscopy level, ultra-structural criteria at the electron microscopy level, and by the production of keratin as determined by polyacrylamide gel electrophoresis, and anti-keratin antibody.

The usefulness of an in vitro model system lies in its ability to mimic the in vivo model. Like intact skin, the epidermal cells have been shown to be both dividing and differentiating. Division was demonstrated by autoradiography, incorporation of the radiactive DNA precursor ^{3}H-thymidine, and increase in cell number. Differentiation was measured microscopically by stratification, Rhodanile blue staining, keratin production and cornified envelope formation.

Optimum growth conditions have been established. The cells are plated at 1.5 x 10^5 cells/cm^2 and grown in Eagle's medium without nonessential amino acids plus 10% fetal calf serum, insulin (1.0 μg/ml) and hydrocortisone (0.1 μg/ml). The cells divide rapidly for several days reaching confluence after two or three days. There is an initial "wave" of keratinization during the first 24 hours, and then continuous keratinization. Following confluence the cells continue to divide and differentiate for one to two weeks. After that time the majority of the cells are terminally differentiated and after four weeks, the cultures completely degenerate.

Feeder layers of irradiated fibroblasts have been used as a substrate for the epidermal cells. The epidermal cells can be inoculated on to these feeder layers at much lower density of 5 x 10^3 cells per cm^2. They then form colonies that do not differentiate as fast or as much as mass cultures. Other substrates such as collagen, and basement membranes are being tried. Growth of the cells in media with reduced levels of calcium markedly inhibits differentation.

The tumor promoter 12-0-tetracecanoyl-phorbol-acetate (TPA) had a dramatic effect on epidermal cell growth and differentiation. When added to the cells, differentiation is measured both morphologically and by cornified envelope formation was reduced by approximately 50%. Also, while the untreated cultures dramatically decreased their rate of DNA synthesis after 2-3 days, those grown in the presence of TPA continued to synthesize DNA as a higher rate for a prolonged time.

Experiments on two-stage carcinogenesis on hamster skin are still in progress. No evidence to date has been obtained to indicate a promotion of DMBA (50 μg) induced tumors by TPA or phorbol didecanoate (PDD).

We feel that hamster epidermal cells in culture represent an excellent system to study differentiation and carcinogenesis. Large numbers of pure epidermal cells can be isolated and when grown in culture these cells undergo a process of differentiation which mimics in vivo epidermal differentiation morphologically and biochemically. Hamster fibroblasts offer the best model system for quantitative studies of neoplastic transformation of diploid cells in culture, because they have a very low rate of spontaneous transformation in the absence of carcinogen treatment but a high rate of transformation following carcinogenic insults. It is possible that these properties are shared by epithelial cells from this species.

Another possible advantage to using hamsters is that our data to date, as well as previous work of others, do not indicate a tumor promoting effect on hamster skin tumors by TPA, which is a potent promoter on mouse skin. Yet, TPA has a pronounced effect on hamster epidermal cells in culture, significantly inhibiting their differentiation. These two observations are being pursued and may provide some insight into the mechanism of tumor promoters. The effects of TPA on epidermal differentiation and hyperplasia in vivo will be examined as well as the metabolic fate of ^{3}H-TPA on hamster skin and hamster cells in vitro.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since skin cancer is one of the most prevalent human neoplasms, and one clearly influenced by environmental factors, finding a model to investigate the complex interrelationships between target cells, carcinogens, and promoting agents, as well as the progresion from normal to preneoplastic to neoplastic cells would be of considerable value to the understanding of environmental carcinogenesis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25001-02 LPFT

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Cellular and Molecular Mechanisms of Neoplastic Progression

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. C. Barrett	Senior Staff Fellow	LPFT	NIEHS
	S. Sheela	Visiting Fellow	LPFT	NIEHS
	E. Elmore	Intermittent	LPFT	NIEHS
	T. Gray	Biologist	LPFT	NIEHS
Other:	E. E. Sisskin	Staff Fellow	LPFT	NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Environmental Carcinogenesis

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 3.75	PROFESSIONAL: 3.75	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to elucidate the cellular and molecular mechanisms of neoplastic development and to understand how environmental agents influence the progression of normal cells to malignancy. The specfic aims of the research are: (1) identification of preneoplastic stages during neoplastic development; (2) determination of the role of somatic mutation in a multi-stage model of carcinogenesis; (3) elucidation of whether benign neoplastic cells progress to malignant cells; and (4) understanding the biochemical basis of cellular invasion.

The studies involve model systems of oncogenesis which employ fibroblasts and epithelial cells in culture.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Fol^+ is a preneoplastic cell line derived from hamster embryo fibroblasts (PNAS, 75: 3761, 1978). Studies with this cell line are done in part in collaboration with B. Crawford and P. Ts'o, Johns Hopkins University. KD cells are normal diploid human fibroblasts and TK cells are a chemically induced neoplastic transformed derivation of KD cells. Both KD and TK cells were the generous gift of Dr. Takeo Kakunaga of the National Cancer Institute.

Papillomas are induced in animals by one of two carcinogenic insults. Weanling hamsters (inbred strain 15.16 or 87.20, Telaco) are injected with 5 mg of diethylnitrosamine either once or weekly for 5-10 weeks. After 8-12 months, a high percentage of the animals develop tracheal papillomas. The animals with such lesions are easily identified by their impaired breathing due to obstruction of the trachea by the neoplasms. The animals are sacrificed and their tracheas removed. Tumors can be recognized macroscopically and prove to be exclusively papillomas by histological criteria. These tumors are excised with or without the cartilage rings of the tracheas and placed in culture with Hams F-12 media supplemented with 10% fetal calf serum.

Epidermal papillomas were induced on the backs of hamsters (inbred strain 15.16 or 87.20, Telaco), by 7-12 dimethylbenzanthrancene (50-400 μg) with or without promotion by phorbol esters. These lesions were excised, minced and placed in culture with various media and supplements. Papilloma bearing Balb/C mice were kindly provided by Dr. Tom Slaga, Oak Ridge National Laboratory.

The three main cell systems which we have selected for studying cellular invasion are: (1) normal, preneoplastic and malignant (chemically transformed) fibroblasts from Syrian hamster embryo cells; (2) carcinoma cells from hamster tracheas (provided by Dr. Brooke Mossman, Univ. of Vermont); and (3) papilloma cells from hamster tracheas.

The invasive properties of these cells are determined by four assays: (1) subcutaneous tumor formation; (2) peri-hepatic tumor formation; (3) peri-renal tumor formation; and (4) infiltration property when inoculated on the chorio-allantoic membrane (CAM) of 8-10 day old embryonated eggs. The first three assays are subjective in nature while the last one can be used to obtain quantitative information about the different cell lines. In addition, the metastatic potential of the cells is determined by intravenous injection and this property can be correlated with invasiveness.

Assays of fibrinolytic activity, collagenase activity and elastase activity are performed by established procedures.

Pulmonary basement membrane from Syrian hamster lungs is isolated and purified by extraction with acetic acid and N-lauroyl sarcosine, and labelled with $^3H\text{-}NaBH_4$ by reductive alkylation for use as an invasion substrate.

MAJOR FINDINGS AND PROPOSED COURSE: The spontaneous transformation of Syrian hamster embryo cells in culture occurs by at least two steps. An intermediate state is represented by an aneuploid cell line (designated Fol$^+$) which is preneoplastic. These cells differ from diploid cells by a number of properties, including their ability to become neoplastic when injected *in vivo* while attached to a substrate. These preneoplastic cells become neoplastic in culture, and growth in soft agar (Aga$^+$ phenotype) appears to signify neoplastic conversion and can be used as a quantitative assay for this transition.

Several results suggest a somatic mutational basis for the Aga$^+$ phenotype: 1) Fol$^+$-Aga$^+$ strains are stably anchorage independent, indicating a hereditable nature of the Aga$^+$ phenotype; 2) A Luria-Delbrück fluctuation analysis demonstrated that Aga$^+$ variants arise spontaneously and at a rate (5.7 x 10^{-8} variants/cell/generation, P_0 calculation) consistent with single locus somatic mutation in these cells (7.8 x 10^{-8} Oua^r mutants/cell/generation. These rates are also in close agreement with the measured rate of mutation at the HPRT locus (1.2 x 10^{-8} HPRT$^-$ mutants/cell/generation) in BP6T cells, a subdiploid cell line derived from the same SHE cell preparation following benzo(a)pyrene treatment; 3) The Aga$^+$ phenotype can be suppressed in cell hybrids. When diploid SHE cells are fused with a highly tumorigenic, subdiploid Aga$^+$ line via polyethylene glycol, hybrid cells are initially incapable of colony formation in semisolid agar. However, upon characterization of isolated hybrid clones after 25 cell doublings, Aga$^+$ cells are evident. Moreover, a wide range of growth efficiencies (Aga$^+$ frequency 10^{-5} to 10^{-1}) is observed among isolated clonal strains this time. These results support a recessive nature of the Aga$^+$ phenotype, with re-expression of anchorage independence in cell hybrids following chromosome segregation or rearrangement.

One of the possible mechanisms for the secondary steps in neoplastic development is that the initial carcinogenic event induces genetic instability, which leads subsequently to secondary changes in a "cascade" fashion (e.g., if the secondary steps are due to mutations, an increased mutation rate would lead to an increased rate of neoplastic progression). Studies on the mutation rate of normal and transformed cells require normal, diploid cells and a transformed derivative of these cells. KD and TK, established by Dr. Kakunaga, NCI, provide such paired cells. We have established growth conditions for these cells which allow a 70-90% cloning efficiency of even the normal cells. Studies are in progress to determine the mutation rates, using a Luria-Delbrück fluctuation analysis, of the two cell types at codominant (Na$^+$/K$^+$ATPase), X-linked recessive (HPRT), and autosomal recessive (APRT) loci.

Although only limited experiments have been performed, attempts to obtain cell cultures from epidermal papillomas have been unsuccessful to date. In contrast, cell lines have been derived from tracheal papillomas with a nearly 100% yield. These cultures have been derived from outgrowths of tracheal explants as well as from excised tumors. Explants of tracheas from untreated animals yielded cell outgrowths but these could only be maintained for a few weeks in culture and could not be subcultured. The normal tracheal outgrowths consisted of polygonal cells with a few ciliated cells. The growths from the DEN treated tracheas appeared morphologically similar to the normal cells with the exception of the absence of the differentiated cells and an increased growth rate. Fibroblasts grow out of the explants after a much longer time than the epithelial cells and could be omitted from the cultures by removing the explants before fibroblast outgrowth occurs. These cells are clearly altered from the normal tracheal cells in their enhanced *in vitro* growth and in this respect, they resemble carcinoma cells derived from hamster tracheas. Experiments are in progress to determine if these cells resemble carcinoma cells in terms of their ability to grow in soft agar and produce tumors in syngenic animals.

Using cloned papilloma cells *in vitro*, we hope to prove (or possibly disprove) that cells from benign tumors are the progenitors of malignant cells. Furthermore, the availability of paired benign and malignant cells from the same clone will be a very powerful tool to determine the difference between malignant and nonmalignant cells. It is hoped that the elucidation of this difference will enable the development of quantitative assays to study the progression of benign cells to malignancy and the influence of environmental agents on this important biological event.

Studies of cellular invasion first require well defined populations of cells. Histological criteria of tumor invasion are generally used to distinguish benign and malignant cells but these criteria are subjective and often difficult to standardize. We have established tumor cell injection techniques which are superior to subcutaneous injection. The cells can be administered by peri-renal injection or peri-hepatic implantation. The tumors that form can be clearly distinguished from the border of the normal tissues and invasion is very evident by these assays. Studies are in progress to determine the invasive properties of a number of cell lines by these assays and to compare invasiveness to metastatic potential and to phenotypic growth properties of the cells.

Invasive carcinomas appear to destroy basement membranes but it is not known if this involves any protease action and whether tumor cells have the capacity to hydrolyze basement membranes. We have established a technique to purify basement membranes from hamster lungs and radioactively label this substrate. A highly malignant fibrosarcoma from hamsters hydrolyzes this basement membrane very extensively. Studies are in progress to determine the mechanism of this degradation and to determine if other malignant tumor cells can degrade basement membranes. Also, studies are being done to determine if this is a proteolytic degradation and if known enzymes like plasminogen activator or collagenase are involved.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
Fifty to ninety percent of all human cancers are environmentally related. Before effective programs in cancer prevention and control can be formulated, a better understanding is required of the mechanisms by which

environmental agents induce and promote neoplasia. Since cancer is primarily a cellular disease, this project utilizes model systems of cells in culture to study the cellular and molecular mechanisms of environmental carcinogenesis. Of particular interest is the cellular development of neoplasia. The progressive nature of neoplastic transformation recently has been recognized but the role of environmental agents in this process is unclear. This problem is central to environmental carcinogenesis and is the focus of this project.

PUBLICATIONS

Barrett, J. C. and Ts'o, P. O. P.: Mechanistic Studies of Neoplastic Transformation of Cells in Culture in Polycyclic Hydrocarbons and Cancer: Environment, Chemistry and Cell and Molecular Biology; ed. P. O. P. Ts'o and H. Gelboin, Academic Press, Inc. (1978).

Barrett, J. C.: Progressive Nature of Neoplastic Transformation of Syrian Hamster Embryo Cells in Culture. Prog. Bxp. Tumor Biol. In press.

Imada, M., Clark, S. M., Fink, L. M., and Barrett, J. C.: Heterogeneity of high molecular weight proteins from transformed and untransformed Syrian hamster cells. Biophys. Biochem. Acta, in press.

Barrett, J. C., Crawford, B. D., and Ts'o, P. O. P.: The role of somatic mutation in a multistage model of carcinogenesis, in "Mammalian Cell Transformation by Chemical Carcinogens," edited by V. C. Dunkel and R. A. Mishra, Pathol. Publishing Co., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25002-02 LPFT

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

In vitro Carcinogenesis and Promotion Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	V. E. Steele	Staff Fellow	LPFT NIEHS
Others:	G. M. Cohen		Surrey, England
	A. C. Marchok		ORNL, Oak Ridge, TN
	P. Nettesheim	Chief, LPFT	LPFT NIEHS

COOPERATING UNITS (if any)
University of Surrey, England, Pulmonary Carcinogenesis Program, Biology Division, Oak Ridge National Laboratory

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Environmental Carcinogenesis

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our major aim in these studies is to study various phases of respiratory tract carcinogenesis at the cellular level using in vitro model systems.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The metabolism of benzo(a)pyrene (BP) to oxidative and conjugated metabolites in tracheal epithelial cells was studied at different levels of tissue organization. Tracheal organ cultures, in which the normal histiotypic organization was maintained, were studied as well as primary tracheal epithelial cultures and a nontumorigenic and a tumorigenic tracheal epithelial cell line.

MAJOR FINDINGS AND PROPOSED COURSE: Short-term organ culture of tracheas, primary cultures of tracheal epithelial cells and the nontumorigenic tracheal epithelial cell line all metabolized ^{3}H-BP in a qualitatively similar way to both ethyl acetate-soluble and water-soluble metabolites. Only the tumorigenic cell line was unable to metabolize BP. The major acetate soluble metabolite formed was 9,10-dihydrodiol with much smaller amounts of 7,8-dihydrodiol and very little if any monohydroxybenzo(a)pyrenes. Enzymic hydrolysis of the water-soluble fraction with β-glucoronidase, released 3-OH-BP, quinones, and 7,8-dihydrodiol as the major metabolites, with smaller amounts of 9-OH-BP and little or no 9,10 or 4,5-dihydrodiol.

Further studies are being conducted on neoplastic transformation of the primary cultures and the non-neoplastic epithelial cell line with BP and some of its reactive intermediates.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies are preparing the way for investigations on the mechanisms involved in transformation of respiratory tract epithelium by environmental carcinogens and some important classes of fossil fuel carcinogens. Respiratory tract epithelium is the major target for such carcinogens in man.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 25003-02 LPFT

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Tumor Induction Studies with the Tracheal Transplant Model

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. Topping	Staff Fellow	LPFT	NIEHS
Other:	P. Nettesheim	Chief, LPFT	LPFT	NIEHS

COOPERATING UNITS (if any)

Pulmonary Carcinogenesis Program, Biology Division, Oak Ridge National Laboratory

LAB/BRANCH
Laboratory of Pulmonary Function and Toxicology

SECTION
Environmental Carcinogenesis

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 2.4	PROFESSIONAL: 1.3	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The fate of carcinogen induced metaplastic-dyplastic lesions induced in airway mucosa was studied. It was found that the great majority of lesions induced by a brief carcinogen exposure is reversible and does not develop into invasive carcinomas.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Rat tracheas were heterotropically transplanted subcutaneously to the backs of isogenic recipients. After the tracheal grafts were fully established, they were exposed for 1, 2, or 4 weeks to pellets containing 165 μg of the carcinogenic polycyclic hydrocarbon DMBA. 40-50 tracheas each were removed at repeated time intervals between 2 and 12 months after the end of exposure and the number of metaplastic-dysplastic lesions was determined. A 29-months tumor incidence study with over 80 tracheas was conducted in parallel (165 μg pellets for 4 weeks).

MAJOR FINDINGS AND PROPOSED COURSE: The peak incidence of metaplastic-dysplastic lesions occurred at 4 months after the end of exposure with 80% of the tracheas having one or more lesions (all types). From that time on the total number of lesions declined steadily to an incidence of approximately 40% at 12 months. Simultaneously the number of lesions per trachea also declined. Only the number of the most advanced dysplasias (severe atypias and carcinoma *in situ*) remained constant. The cumulative incidence of invasive carcinomas at 29 months was near 10%. The data clearly indicate that without further exposure (to either a carcinogen or a promoting agent) most of the metaplastic-dysplastic lesions induced by a brief and modestly strong carcinogenic insult, are reversible and do not progress to invasive carcinomas. From studies with (the phorbol ester) TPA and with asbestos fibers, it may be inferred that further exposure of the lesions will "promote" them to develop into invasive carcinomas. Whether the histologically recognizable metaplastic-dysplastic lesions are truly precursors of cancers is, however, called into question by this and other studies in our laboratory. This problem is being further investigated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A problem of great theoretical and practical importance in environmental carcinogenesis is whether morphological or other markers exist to signal the appearance of preneoplastic cells, i.e., cells that are precursors of cancer. In the respiratory tract mucosa of humans and animals exposed to products of incomplete combustion of organic materials and to other carcinogenic substances, histopathological alterations have been described which are thought to represent precursors of neoplasias.

Whether such lesions are indeed reliable signals foreshadowing the appearance of cancer, is of great consequence in dealing with humans at high risk to develop lung cancer. It is also of great significance for the development of short-term *in vivo* bioassays. It has been proposed that putative preneoplastic lesions might be used as endpoints for carcinogenesis bioassays.

UNITED STATES ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION
OAK RIDGE OPERATIONS
Oak Ridge, Tennessee 37830

TITLE: Susceptibility of Preneoplastic Epithelial Lesions to Toxic and Carcinogenic Insults.

CONTRACTOR'S PROJECT DIRECTOR: A. J. P. Klein-Szanto, Ph.D.

PROJECT OFFICER (NIEHS): Paul Nettesheim, M.D., Chief, LPFT

DATE CONTRACT INITIATED: October 1, 1978

CURRENT ANNUAL LEVEL: $165,000

PROJECT DESCRIPTION

OBJECTIVES: During the last year we have characterized the lesions produced by a short exposure to DMBA in heterotopic rat tracheal transplants. The lesions were characterized according to their histopathological, ultrastructural, histochemical, cell kinetics, and morphometric features. The following lesions, first classified using histopathological criteria alone, were studied: atrophic epithelia, squamous metaplasia without atypia, squamous metaplasia with atypia (in three variants with mild, moderate, and severe atypia) carcinoma in situ, and invasive carcinoma. Although many structural features and quantitative characteristics of the different lesions were similar, several specific features were found in each of the epithelial alterations. The morphometric and cell kinetics data revealed a gradual change of the numerical values, in the same direction, as the lesions became more atypical.

METHODS EMPLOYED: Heterotopically transplanted rat tracheas which were continuously exposed to measured amounts of DMBA for four weeks were embedded in Epon and processed for light microscopical (toluidine-blue), histochemical (PAS and alcian blue), and electron microscopical observation. Most of the transplant bearing animals were also injected with tritiated thymidine prior to sacrifice. This enabled us to perform autoradiograms from the Epon embedded material. A morphometric study of the 1-2 μ thick Epon sections of precursor lesions was carried out using a planimetric graphics tablet connected on line with a computer. A specially devised program calculates a series of cell and nuclear size indicators. The parameters which we have analyzed are: mean cell surface, mean nuclear surface (indicators of cell and nuclear size) and the nucleus over cytoplasm ratio.

MAJOR FINDINGS AND PROPOSED COURSE: Nondysplastic hypotrophic and metaplastic epithelial alterations induced by DMBA in isogenic tracheal transplants were studied by light and electron microscopy 3 to 24 months after cessation of a 4-week carcinogen exposure. Hypotrophic epithelium observed at all timepoints was characterized by the presence of non-ciliated cells which adopted either cuboidal or squamous shapes forming simple or bistratified epithelia. Most of these cells as well as some metaplastic cells exhibited features of mucin secreting cells. The metaplastic epithelia included non-keratinizing squamous metaplasia, closely related to transitional metaplasia and keratinizing squamous metaplasia, which presented either an atrophic or an acanthotic epithelium. Although many of these epithelia showed morphological features of normal stratified epithelia, several non-keratinizing squamous metaplasias and acanthotic keratinizing squamous metaplasias exhibited some irregularities, probably representing very early atypical ultrastructural features, i.e. perinuclear concentration of tonofilament-bundles, presence of dark and clear basal epithelial cells, interruptions and alterations of the basal lamina. These features were not observed in a group of early squamous metaplasias studied for comparative purposes two weeks after cessation of DMBA exposure, which were characterized by a combination of degenerative phenomena and increased cell proliferation.

In addition to the non-dysplastic lesions, a series of dysplastic alterations including squamous metaplasias with various degrees of atypia, carcinomas _in situ_ (CIS) and microinvasive carcinomas (CA) were studied by light and electron microscopy in the same material. Most of the lesions appeared between 16 and 48 weeks. Although most of the pathological characteristics could be observed in all the lesions under study, an increasing gradient of morphological and ultrastructural dysplastic alterations from the mildest to the more advanced states was noted. An addition to the classical nuclear and cytoplasmic indicators of atypism, the squamous metaplasias with slight and moderate atypia were characterized by the presence of glycogen in the spinous layer, atypical composite keratohyalin granules in the granular layer and in most cases an orthokeratinized horny layer. The squamous metaplasias with severe atypias showed an increased secretory activity and a decreasing number of desmosomes. Keratinization of the surface layer was rarely seen. Carcinoma _in situ_ was characterized by numerous intra- and extracellular microcysts filled with secretory material identified by histochemical techniques as containing neutral glycoproteins and sialomucins. Microinvasive carcinoma showed similar characteristics, with an increase in polylobulated nuclei and appearance of multilaminar ergastoplasm. An outstanding feature in all these lesions was the presence of dark fusiform epithelial cells. Confined to the basal layer in the mildest epithelial changes, they were observed in the middle layers in the squamous metaplasias with severe atypia, and appeared in all layers of the carcinoma _in situ_.

The labeling index (LI) of the basal layer showed a gradual increase from 16 ± 5 (%) in the regular metaplasias without atypia to 31 ± 2 for CA and 32 ± 7 for CIS. The atypical squamous metaplasias exhibited a LI of approximately 19. Whereas the suprabasal layers' LI was of 2 ± 2 in the nondysplastic squamous metaplasias in all other lesions starting with the moderately dysplastic squamous metaplasia it was higher than 6 reaching a maximum value of 10 in CIS.

The use of computerized techniques of morphometric analysis allowed the study of numerous cell and nuclear parameters, i.e. nucleus/cytoplasm ratio (N/C) and various shape parameters. The group of lesions under study included, regular squamous metaplasia, squamous metaplasia with moderate atypia, squamous metaplasia with severe atypia, carcinoma in situ and invasive carcinoma. Control tissues included, normal respiratory epithelium and normal esophageal epithelium. Each group of lesions and normal epithelium consisted of 4 to 16 different cases. Cells were sampled in column from the base to the surface layer (excluding keratinized cells) and divided into sets of cells i.e. basal cells (those in immediate contact with the basement membrane), surface cells (those in immediate contact with the lumen or the last non-keratinized cell) and middle cells (all the rest). In each lesion or normal epithelium, the sample of a said set of cells consisted of 200 to 1000 cells. The sampling of CA cells was somehow different and the cells were categorized into two sets of cells, i.e. basaloid (poor differentiation and in immediate contact with the basement membrane) and spinous (spinous differentiation). The N/C ratios permitted a clear differentiation of the surface cells. The regular squamous metaplasia exhibiting a mean N/C=0.07 and the CIS a mean N/C=0.50, whereas the other lesions showed intermediate values. The esophageal epithelium showed similar values as the regular squamous metaplasia. The middle set of cells also showed statistically significant differences between most epithelia understudy. The N/C of the basal cells was very similar in all groups except CIS and CA which showed N/C values around 1, whereas the other lesions exhibited values of approximately 0.70.

Various shape parameters were also analyzed, showing interesting changing patterns between the different epithelia, especially a clear quantitative difference was detected in the shape of surface cells, a phenomenon closely linked to the different degrees of keratinization observed in the various groups of lesions.

Having characterized the lesions produced in our experimental model, we are ready to proceed with the study on the influence of various agents on the evolution of preneoplastic lesions. The first experiment now being prepared involves the establishment of a two-stage carcinogenesis model in which very low doses of DMBA will be used in the initiation phase, followed by TPA delivered continuously during a longer promotion phase. The experiments will be carried out in tracheal transplant using intratracheal pellets containing beeswax and cholesterol as vehicle and either the carcinogen or the promoter.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The purpose of the studies is to develop a better understanding of the morphological and biological characteristics of putative preneoplastic lesions in respiratory tract epithelium. They will provide information on the environmental and host factors which govern the progression of preneoplastic changes to invasive carcinomas. This knowledge if essential to design, in the future, rational approaches for intervention therapy in groups of individuals at high risk to develop cancer. This work will also provide data which will help to decide whether preneoplastic lesions can be used as endopoints in short term *in vivo* carcinogenesis bioassays.

RESEARCH RESOURCES PROGRAM

RESEARCH RESOURCES PROGRAM
Summary Statement

The Research Resources Program provides the principal, technical, and scientific support capabilities to the Intramural Research Programs of the NIEHS. It provides scientific support in the areas of chemistry, comparative pathology, inhalation toxicology, laboratory animal medicine and husbandry, and research contracts coordination; engineering support through facilities planning, operation, and maintenance; as well as technical support through the operation of the NIEHS, NIEHS safety program, arts, graphics and photography, as well as glassware and media supply.

The Research Resources Program also plans, develops, and conducts research in targeted areas. This is accomplished independently or in collaboration with other NIEHS laboratories, Federal agencies, academic institutions, or contract laboratories.

RESEARCH CONTRACTS

The NIEHS Research Contracts Program reflects an extension of Intramural Laboratory capabilities and interests. The Research Contracts Office coordinates the allocation of contract funds, peer review of contract experimental design, evaluation of contract proposals, and the monitoring of contracts upon award. A total of $6.4 million was invested in over 40 research contracts in FY 79. Three-fourths of these contract funds were allocated to the National Toxicology Program. The quality and effectiveness of all research contracts is now considered by the NIEHS Board of Scientific Counselors who review research contract activities as part of their evaluation of intramural research activities.

NIEHS LIBRARY

The NIEHS Library is the principal science reference resource for the Institute. Library and information services include manual and computerized literature searching of more than 100 bibliographic data bases, maintenance of a collection of some 450 journal titles and 3500 books on environmental health, participation in a nation-wide network for interlibrary loan and cataloging purposes, procurement of 1800 new books for the Library and the laboratories, and compilation of the annual bibliography of publications by Institute personnel.

Reference/Literature Searching: The Library maintains one of the most up-to-date computerized literature searching capabilities in the world, with access to more than 100 data bases covering subjects from business administration through toxicology. During FY 79, Library personnel performed searches on 800 topics, usually using several data bases per question to ensure complete coverage. The number of regularly-scheduled current awareness searches (SDI's) continued to increase, but the printed copy of Current Contents remains the principal alerting service used by Institute personnel.

A new data base, the Toxicology Data Bank (TDB), is unique among the others in offering hard data instead of bibliographic references. TDB, provided by the National Library of Medicine, gives comprehensive toxicological information about some 1,000 chemicals. It should be closely associated with the research of the National Toxicology Program.

Journal Collection: The journal literature continues to be the principal means of disseminating scientific information. Consequently, the Library emphasizes its journal collection to try to cover as many of the diverse subject areas as possible which fall under "environmental health".

The Library subscribed to 90 new journals during FY 79, bringing the total to more than 450. In addition, the Library ordered about 75 journals for the various laboratories. A small branch collection of duplicate titles was established in the Northrop Building for the convenience of off-site personnel. The Library continues its policy of selectively binding journals or replacing them with microfilm to save space. By participating in several exchange programs, many missing issues have been replaced.

The NIEHS Library, working with EPA, produced a computer-generated journal holdings list updated for 1979.

Book Collection: Reflecting the decision to develop the book collection more thoroughly, 1800 books were purchased by the Library in FY 79. This was a 50% increase over the preceding year. About half of the books were purchased for the Library and about half for the Branches.

Working in association with the Comparative Medicine Branch Chief, the Librarian concentrated on selecting books on laboratory animals and veterinary medicine. Also, the mutagenesis collection in Building 7 was merged into the main collection.

By joining the Federal Library Committee, the Library obtained access to an automated cataloging system, OCLC (the Ohio College Library Center). OCLC is a computerized union catalog of books held by more than 1,000 libraries nation-wide. The NIEHS Library has experienced a tremendous savings in time owing to the 95% hit rate for our new books which already have cataloging data on OCLC. At a push of the button the Library can order cards for the card catalog.

As an additional benefit of participation in OCLC, the Library gets a monthly print-out of the new books it has cataloged. This forms the core of the Library's newsletter and acquisitions list.

Another product from OCLC is a computer tape of the Library holdings. In another joint project with the EPA Library, the NIEHS Library is developing a Computer-Output-Microfiche. The print-out will be reduced, duplicated, and bound; then copies will be distributed to Institute personnel so they will be able to tell at a glance whether or not the Library holds a given book. This system will present the capability of being maintained online for looking up books on remote terminals. The target date for full implementation is June, 1980.

Interlibrary Loan: Significant progress has been made in acquiring interlibrary loans and photocopies. First, about 10,000 requests were made in FY 79, 10% fewer than in FY 78, reflecting the improved in-house collection. Also, 30% of those requests were filled by the Library from our own collection. Another 25% were filled by a student employed at NIEHS to do photocopying work at the Duke Medical Library. Less than half the requests had to be sent to other libraries, the main one being N. C. State University.

An investigation into alternative arrangements with the NIH Library was made, but the procedure with the Duke Medical Library was deemed preferable.

The OCLC computerized cataloging system, mentioned above, also proved useful for verifying titles for interlibrary loan and for locating holding libraries from which to borrow books. An interlibrary loan subsystem designed to cut-down on paperwork and time was made available midway through the fiscal year but has yet to prove its utility.

Manuscript Collection/Annual Bibliography: In FY 79 the Library took over responsibility for the collection of manuscripts of papers authored by NIEHS personnel. With the aid of the Biometry Branch, an automated system for keeping track of them was developed. The system permits replacement of manuscript records with reprint records when an article is acutally published. These reprint records are then used to generate the annual cumulative bibliography of NIEHS publications.

Planning: Planning for library and information services to meet the needs of an expanded organization and new facility got underway in FY 79. Having the Institute personnel separated into two campuses presents the need for at least two reference collections; in actuality there will be several branch libraries in the new facility. The Librarian met with the individual Lab Chiefs to discuss their needs in order to make the best use of the space in branch libraries. In contrast to the views of the Lab Chief, many of the bench scientists expressed a desire to have the main collection in the new building. Alternative plans were drawn up and ways of carrying them out are currently being explored.

In addition to the various services mentioned above involving automation, the Librarian investigated computerized circulation systems, acquisitions systems, and serial check-in systems. In conjunction with the Comparative Medicine Branch Chief, the Librarian also investigated the Laboratory Animal Data Bank for its possible use at NIEHS.

In December the Librarian completed research on user attitudes about priorities for library services. This involved a survey of 1200 scientists at NIEHS, EPA, and Burroughs Wellcome, Co. From the results, a model was formulated to be used for planning or evaluating specialized research libraries. Journal purchases, computerized literature searching, interlibrary loan, and book purchases were the services given top priority by the users. The Librarian reported these findings and described the model in a paper presented at the 70th Annual Conference of the Special Libraries Association.

OFFICE OF SAFETY

The Office of Safety includes three qualified, experienced safety and health personnel. Major advances of the office include:

Safety Plan: A safety plan for the Institute was developed based on an implementation of the newly written NIEHS Safety and Health Manual and newly revised Radiation Safety Guide. Aspects of the plan include: appointment of Laboratory or Branch Safety Coordinators; establishment of medical emergency procedures, institution of Emergency Reaction Teams; development of a system requiring safety protocols for handling radioisotopes, hazardous chemicals and biohazardous agents; coordinating the medical surveillance effort; providing for adequate waste disposal; and Stregthening the safety training program.

Safety and Health Manual: The Manual was completed by the NIEHS Safety Committee and Safety Office and approved by the Intramural Council, NIH Safety Office and Publication Clearance Office. The Manual has been issued a DHEW (NIH #79-1848) and is scheduled for printing by NIH. A limited number of copies (50) were distributed to all Laboratory, Branch Chiefs and Safety Coordinators. The Manual is in loose leaf form with dated pages, contains 14 tabbed sections for easy, rapid use and is indexed. In addition to comprehensive safety information, included are appropriate safety forms (e.g. protocols, new employee check lists, waste disposal, accident), policy, procedural and individual responsibility statements and an up-to-date reference section subdivided by category. Efforts have been coordinated with the Library to insure references are available in the Library.

Radiation Safety Guide: This manual has been completely revised by the Radiation Safety Officer, approved by the Radiation Safety Committee and issued to all radioisotope users. The format is now loose-leaf and conforms to the Safety and Health Manual.

Safety Coordinators: Safety Coordinators have been appointed for every Laboratory and Branch and major organizational units at the Institute (19). Their duties include: assisting in the implementation of the Safety Manual; interacting with their groups in matters of general safety and promoting safety awareness; periodically accompanying the safety officers on safety inspections; assisting in the completion of safety office forms (e.g. protocols, new employee check lists, etc.); suggesting special training needs; interacting with the Office of Safety; and aiding in the screening of purchase orders for hazardous agents.

Medical Emergency Procedures: A plan has been implemented to provide a central phone number, which is always manned during working hours, to receive and respond to medical emergency reports. When a medical emergency is ascertained by the received of the call, a response in the following order takes place: voice page NIEHS M.D. on call and state emergency location; call ambulance (rescue unit) if needed; call guard desk to dispatch medical emergency equipment (defibrillator, oxygen, emergency medical kit), voice page safety personnel to provide CPR, first aid, assist M.D., control crowds, and meet ambulance. To aid in facilitating these procedures all Institute phones have red stickers listing appropriate emergency phone numbers.

Emergency Reaction Teams: Two-person emergency reaction teams have been set up in the 19 buildings on the NIEHS Campus, the warehouse, trailer city, Northrop, Extramural Programs located in Raleigh (North Hills) and are planned for the new facility. Appointed team members are trained in cardiac pulmonary resuscitation (CPR), first aid and use of fire fighting equipment. Additional duties include, when feasible, insuring complete evacuation of building employees in appropriate emergency situations.

Safety Training: Routine, periodic training programs conducted by safety office personnel include General Laboratory Safety (9 hours quarterly); Introductory Radiation Safety (8 hours quarterly); Proper Use of Fire Fighting Equipment with individual practice and use of equipment (3 hours, semi-annually); Fundamental of Fire Prevention (1 hour quarterly, in coordination with General Lab Safety). The Office of Safety provides for monthly safety training of Warehouse and Research Services Branch personnel and quarterly training sessions for Comparative Medicine Branch personnel. Contract training has been scheduled in certified CPR (four times a year with follow-up yearly recertification planned) and certified first aid (four times a year with three year follow-up recertification). Several investigators have taken intensive one-week radiation safety training at NCSU and three-day courses in Safe Handling of Chemical Carcinogens and Biological and Injury Control in the Biomedical Laboratory.

Protocol Concept: The hazardous agent protocol concept has been instituted for research involving use of hazardous chemicals, radioisotopes and biohazardous agents. As part of this program the Safety Officer and Radiation Safety Officer screen all purchase orders for hazardous agents. (When feasible, safety coordinators assist to a limited degree.) Further checks and balances being developed to implement this system include medical surveillance programs, periodic safety inspections, inventories and waste disposal request forms.

Medical Surveillance: Personnel using or exposed to hazardous agents or other occupational risk situations are scheduled for baseline medical exams as well as follow-up exams (usually on a yearly basis). Every attempt is made to schedule the initial exam before work begins. Medical programs for issuance of prescription and non-prescription safety glasses as well as audiometric exams are also in force.

Inspections: Unannounced quarterly inspections of all Institute areas are conducted under a new system. The Safety Officer is accompanied by the safety coordinator (or a representative) of the Research Services Branch, at least twice yearly by the radiation safety officer and the safety coordinator of the area being inspected is invited to accompany the inspectors. The results are direct communication, time reduction for work requiring repairs or changes and improvement of cooperation.

Environmental Monitoring: The limited monitoring program for potential workplace hazards is being maintained. The chemical synthesis high hazard room in Building 2 is being monitored for specific compounds, by contract, as part of a trail program. An assessment with prioritization is being made for areas in need of monitoring.

Waste and Disposal: Institute chemical waste is no longer shipped to NIH for disposal. A contract is being let for an appropriate, certified and approved organization to pick up Institute waste from the campus. This is already done for radioactive waste (except scintillation vials and contents) by Chem Nuclear with the vials picked by Rad Services. A more unified approach is not currently possible. A safety task force has been appointed to investigate proper technology for on-site chemical deactivation of hazardous waste and identification of an appropriate state-of-the-arts incinerator for the new facility. The small incinerator on campus for non-hazardous waste is now scheduled for yearly inspections and certification.

Safety Committee: The Office of Safety assisted the Safety Committee in its functions which included: development of a procedure for accident investigation, investigation of technology to be used for monitoring of areas where hazardous chemicals have been used (with emphasis on the Building 14 high hazard room) and monitoring non-laboratory areas where use of organic solvents could cause personnel exposure problems.

Miscellany: Surveys were conducted on the Research Services Branch, Warehouse, Chemical Storage Area and Comparative Medicine Branch with recommendations for safety improvement, containment, personnel protection and training.

The Office of Safety is also working with the Biometry Branch to computerize protocols, records, and information on radioisotope inventories and exposure dose.

The Safety Office presented two papers on the protocol concept and associated chemical synthesis laboratory, and the Building 14 high hazard room and proposed DHEW guidelines for handling carcinogens at the American Chemical Society/Chemical Society of Japan Chemical Congress in Honolulu. The Radiation Safety Office presented a paper at the Bioelectromagnetic Society Meeting in Seattle, Washington, on the effect of microwave radiation on quail embryos and a paper at the Washington, D. C., National American Chemical Society Meeting on the Integration of Radiation and Chemical Safety at NIEHS.

Radiation Safety: In addition to revising the Radiation Safety Guide, the Radiation Safety Officer assisted in all of the programs outlined above. Particular emphasis is being spent on coordinating the Safety and Radiation Safety Officers' functions so that they always complement and may temporarily, effectively substitute for each other when ncecssary. Increased use is being made of the Safety Coordinators in renewal and application for radioisotope use protocols. The Radiation Safety Officer is continuing to review all applications for radioisotope use and investigate accidents. About 350 shipments of radioisotopes were received and screened for contamination and leakage this year. The activity was 49% greater than the preceding year indicating an increased use of radioactive materials. More than 1.2 Ci of activity was received this year.

COMPARATIVE MEDICINE BRANCH

COMPARATIVE MEDICINE BRANCH
Summary Statement

The Comparative Medicine Branch (CMB) of the NIEHS programs and coordinates experimental animal procurement, housing and utilization for the Institute; advises Institute scientists of appropriate animal models for use in research programs; maintains laboratories in microbiology, experimental surgery, laboratory animal medicine and mammalian reproduction; and plans and conducts research appropriate to these laboratory functions.

Administration

Personnel: There are three veterinarians, one microbiologist, one animal husbandman, one secretary, two clerks, six biological laboratory technicians, seventeen biological aids, five wage grade laborers and ten students.

The greatest changes in personnel this year were the replacement of two veterinarians, A. G. Edward, D.V.M., Chief of CMB (new position) and Charles A. Harper, D.V.M., replaced Dr. Robert Faith as technician training officer and clinician. Jean Gordner joined the organization as secretary (new position) and Caroline Morris as clerk-typist replaced Ruth Couchman.

Mr. Elton Yancey was promoted to Industrial Washer Systems Operator Foreman from Lead Aid to fill position vacated by Mr. Clyde Rogers.

All positions were audited this year resulting in rewriting a number of job descriptions and grade levels. This exercise created the greatest lift in morale of any one act during this period of development. Vacancies exist for three new positions that are presently being advertised: a) Supervisor Biological Laboratory Technician; b) Surgical Technician; c) Biological Aid.

Space: One of the most critical areas of concern for NIEHS has been space sufficient to maintain the research animals requested, breeding colonies to supply dated pregnancies, and those exotic species not commercially available. This pressure of need in finite space clearly threatened maintenance of animal care standards and indicated the need to construct or otherwise obtain additional animal holding areas.

A plan for construction of an addition to Building 15 was scrapped when it was found that the bid cost was twice available funds and the time for occupancy was the same date as the availability of space in the new South Campus.

A search was made for any animal space available within a ten mile radius of the present facility. Two areas were found that had space designed for animal facilities that were available for lease. Becton-Dickinson leased

NIEHS 720 sq. ft. of space to be used for experimental animal housing and Duke University leased 6,320 sq. ft. of space to be used as a normal animal breeding unit to house dated pregnancies and lines not commercially available. The Duke facility affords the separation of these critically needed lines of animals, that must be kept healthy and free of variables, from animals under experiment. It is scheduled for occupancy July 1, 1979.

However, the addition of 7,040 sq. ft. of space puts NIEHS into position to maintain all the animals required, as forecast at this time, until the opening of the new facility.

Programs: A new contract was let to caesarean rederive F344 rats, C57BL/6 and DBA/2 mice plus other inbred mice as indicated by NIEHS programs. The successful bidder was Harlan Industries of Indianapolis, Indiana.

This will assure a single source of supply of gnotobiotic strains. The lines initiated are those that are most commonly used and not always available from other sources.

The development of new programs for data systems for animal procurement has increased the efficiency of record-keeping, billing and man hours used by 75 percent. Plans are underway to create data systems for the animal census report, equipment and supply inventory, breeding colony reports, animal weighing and feed and water consumption.

Currently, the Animal Husbandry Section's emphasis has been in improving the animal care and technical support, training and development of the biological aids. The increased demand for the technical assistance has resulted in assigning biological aids to work directly with investigators requesting long-term support in the animal rooms and laboratories.

Certification of the biological aids by the American Association of Laboratory Animal Science is an on-going process. To date, there are five (5) Laboratory Animal Technicians, three (3) Assistant Laboratory Animal Technicians and one (1) Technologist certified. Currently, two (2) are seeking certification at the assistant level, one (1) at the technician level and three (3) at the technologist level.

The American Association for Accreditation of Laboratory Animal Care visited and reviewed the laboratory animal care facilities and procedures at the National Institute of Environmental Health Sciences in November. The site-visitors "commended the NIEHS for a well organized and managed laboratory animal resources program." The end result was continued accreditation.

A training officer was acquired in September. Programs are being developed to train the employees in basic reading, writing and mathematics skills, gnotobiotics, sanitation, sterilization and procedures for new animal arrivals.

The planning for the animal facilities on the South Campus are underway. Teams have been established to order equipment, identify personnel, develop traffic patterns and standard operating procedures for each module. Equipment has been ordered for approximately one-third of the animal holding rooms.

The Microbiology Laboratory is primarily a service function. Research is performed when time permits and involves two general areas:

1) Diagnostic or Disease Related Research: Investigation of current disease problems in animals at NIEHS and improving and expanding diagnostic capabilities to cope with these problems.

2) Institute Related Research: This research is geared toward determining "The Effects of Environmental Chemicals on Host Resistance to Infectious Agents." Primary objectives are to develop test systems which determine the effects of environmental pollutants on disease resistance of laboratory animals as measured by mortality rates subsequent to exposure to infectious agents. When increased mortality rates occur, additional studies will be performed to establish why the chemically exposed animals had higher mortality rates as compared to controls.

Research activities in the Microbiology Laboratory have been reduced due to additional duties assigned to the microbiology staff. These duties include:

1) Vermin Control Program: For the first time NIEHS has an outlined vermin control program. The CMB has hired a Stay-in-Schooler to assist the certified pest specialist in carrying out the program "in house" rather than on contract. This will allow NIEHS to have a more effective and less expensive vermin control program.

2) National Toxicology Program Involvement: The NTP is an organization created from components of the National Center for Toxicological Research, the National Cancer Institute, the National Institute of Occupational Safety and Health, and the National Institute of Environmental Health Sciences. It is devoted to the testing of environmental chemicals of public health concern.

A committee was formed and a one-day workshop was held at NIEHS to evaluate the microbiological quality control procedures presently in use at the cooperating Institutes. The following program is recommended: 1) Health Surveillance - test animals should be monitored prior to and at intervals during the study to determine their microbiological profiles; 2) Environmental Monitoring - a) to evaluate routine sanitation procedures, b) feed, bedding, water and accessory equipment should be monitored for microbial quality, and c) sterilization equipment should be routinely monitored with biological indicators; 3) Quality Control within the Microbiology Laboratory - a) sterility checks and media function studies to assure the accuracy of microbiological results, b) a continuing education program consisting of unknown specimens submitted to the cooperating laboratories to assure that all microbiological results are standardized and meaningful; and

4) Interpretation and Reporting of Microbiological Results - microbial data should be computerized thus providing a standard reporting system for permanent storage and immediate retrieval of data which can be integrated with other project data. The effects of microorganisms on animal test systems should be considered when interpreting toxicity test results.

In conclusion, these Standard Microbiological Quality Control Procedures when performed accurately by all cooperating Institutes will assure that the definition of microbiological quality control will be clearly understood and meaningful to the National Toxicology Program.

3) Increased Work Load: The Microbiology Laboratory is receiving more specimens for mycoplasma culture due to an epizootic of mycoplasmosis. This has resulted in more rats being necropsied and at times 3 specimens (lung wash, trachea and nasal or oropharyngeal swab) are submitted per animal instead of one.

The addition of the new Stay-in-School position will relieve some of these problems created by the mycoplasma incident and additional work load.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30008-08 CMB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
The Effects of Environmental Chemicals on Host Resistance to Infectious Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. E. Thigpen	Microbiologist	CMB NIEHS
OTHER:	E. E. McConnell	Veterinary Pathologist	EBB NIEHS
	J. A. Moore	Supervisory Veterinary Medical Officer	RRP NIEHS
	G. A. Boorman	Veterinary Pathologist	EBB NIEHS

COOPERATING UNITS (if any)
Animal Husbandry Section, CMB
Joe Haseman, Ph.D., BB
Histology Section, EBB

LAB/BRANCH
Comparative Medicine Branch

SECTION
Microbiology Laboratory

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.6	OTHER: 0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Previous studies have shown that mice exposed to TCDD (5ug/kg body weight) once weekly for 4 weeks, are more susceptible to infection, and that 5-day Thioglycollate induced peritoneal exudates contain significantly less lymphocytes and macrophages than controls. Present investigations are geared to determine whether this reduction in peritoneal cells is due to impaired chemotactic function or due to an antiproliferative effect on pro monocytes or macrophages. Either effect could contribute to increased susceptibility to infection with Salmonella bern.

These studies were performed as a blind study. Experimental data has not been completely summarized; therefore, the identity of the treatment groups will not be revealed until data is completely summarized. Final results will be completed as soon as possible.

PROJECT DESCRIPTION

METHODS EMPLOYED: (Blind Study) Four-week old male C57BL/6JFH mice were randomly divided into 2 groups. Each mouse was weighed and dosed *via* gavage once each week for 4 weeks with TCDD in acetone-corn oil or with *only* acetone-corn oil. Two days after the last dose of TCDD, a leukocytic exudate was induced in the peritoneal cavity with 2.0 of thioglycollate. Mice were sacrificed at 0, 3, 6, 12, 24, 48 and 120 hours and heart blood, peritoneal exudates, and bone marrow cells were collected for total and differential cell counts. Macrophages were tested for chemotactic function.

MAJOR FINDINGS AND PROPOSED COURSE: These studies have been performed as described above. At the present time, the experimental data has not been completely summarized. Therefore, the *identity* of the groups have not been revealed. When the data is completed for each group, identity will be revealed and results and findings will be evaluated. These studies will continue.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A variety of environmental chemicals when administered in sublethal doses are immunosuppressive and increased mortality rates have been observed upon exposure to infectious agents. These findings are significant in that humans may be exposed to these same compounds in minute quantities and, therefore, may be rendered more susceptible to certain types of infectious agents. It is important that we establish *why* mice exposed to TCDD are more susceptible to bacterial infections.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30078-02 CMB

PERIOD COVERED
July 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Health Surveillance of Laboratory Animals at NIEHS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. B. Feldman	Veterinary Medical Officer	CMB NIEHS
OTHER:	B. N. Gupta	Veterinary Medical Officer	EBB NIEHS
	J. E. Thigpen	Microbiologist	CMB NIEHS
	G. A. Boorman	Veterinary Pathologist	EBB NIEHS

COOPERATING UNITS (if any)
Animal Husbandry Section, CMB

LAB/BRANCH
Comparative Medicine Branch

SECTION
Veterinary Medicine

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	1.2	2.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Incoming and resident animals at NIEHS are monitored for morphological abnormalities, spontaneous diseases and parasitism. The health status of research animals is vital to reliable interpretation of experimental data.

Sporadic respiratory disease among laboratory rodents is associated with murine respiratory mycoplasmosis and Sendai virus. Enteric pathogens have not been detected. Pinworm infections are infrequent. No pathologic lesions can be attributed to these nematodes.

Pasteurellosis in NZW rabbits has not been encountered since purchasing them from a new source. Infectious disease in guinea pigs or hamsters has not been encountered.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: All species and strains of research animals at NIEHS are routinely observed for clinical signs of disease.

New species or strains of animals from new sources undergo complete monitoring, including examination for respiratory and enteric pathogens, ecto- and endoparasites and incidental pathology. Reference histological slides are kept on each strain or species. Pooled sera are periodically screened for viral serology.

Rodents in breeding colonies are surveyed monthly, other resident animals are monitored approximately every 8 weeks for respiratory pathogens and parasites. Tissues from major organs are collected for histopathologic examination. Fecal samples are collected weekly from individual shipping crates and examined by the flotation method for parasite eggs. Positive results confirm infestation at the supplier.

Dead, diseased or abnormal animals are usually submitted to necropsy after clinical examination.

MAJOR FINDINGS AND PROPOSED COURSE: Incoming and resident rats monitored at monthly intervals have been free of salmonellosis. An epizootic of murine respiratory mycoplasmosis, etiology unknown, necessitated decontamination and restocking of three experimental and one breeding rat rooms. The cause of the epizootic is being investigated and measures are being explored to prevent a recurrence. Occasionally, newly received weanling rats revealed focal corneal opacity and periorbital edema. This condition has proved to be resolving stages of sialodacryoadenitis, a transient viral infection. Lesions usually disappear completely within a week.

Swiss and inbred mice have been relatively free of respiratory infections. Variable titers to Sendai virus are encountered but lesions attributable to this respiratory virus are uncommon. Pinworm infections are sporadically encountered but clinical cases of diarrhea or prolapsed rectum have not been seen. The presence of this parasite is esthetically displeasing but eliminating the infection is extremely difficult to accomplish. Hamsters have been relatively disease free. Guinea pigs purchased from a reliable SPF colony have been free of infectious disease. The incidence of "snuffles" (pasteurellosis) in NZW rabbits has virtually been eliminated since changing source of supply. During the period of 6 months (October, 1978 through March, 1979) 546 animals were necropsied for health surveillance. Surveillance of laboratory animals will continue on a routine basis.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The health status of all research animals must be continually monitored; otherwise, intercurrent infections could cause misinterpretation and possible negation of valuable experimental data. In addition, microbiological and histopathological surveillance has uncovered interesting and valuable data heretofore unpublished in laboratory animal medicine.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 ES 30080-02 CMB

PERIOD COVERED
July 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Simplified Gastric Intubation in the Rabbit

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. B. Feldman	Veterinary Medical Officer	CMB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Comparative Medicine Branch

SECTION
Veterinary Medicine

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL: 0.5	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Gastric intubation is frequently employed in laboratory animals as a method of administering precise doses of liquids. This technique is difficult in the rabbit because of limited oral space. A technique was developed to simplify this procedure by utilizing a 13 gauge curved stainless steel feeding needle which is bent to approximate the curvature of the rabbit throat. By using a rigid tube that has been properly shaped, the likelihood of entering the trachea is minimized.

PUBLICATIONS

Feldman, D.B.: Simplified gastric intubation in the rabbit. *Lab. An. Sci.* *27*: 1037-1038, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30081-02 CMB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
The Effect of Dietary Protein on Lifespan, Frequency of Nephritis and Degree of Hypertension in the Golden Syrian Hamster

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. B. Feldman	Veterinary Medical Officer	CMB NIEHS
OTHER:	E. E. McConnell	Veterinary Pathologist	EBB NIEHS
	J. Knapka	Nutritionist	VRB NIH
	G. A. Boorman	Veterinary Pathologist	EBB NIEHS

COOPERATING UNITS (if any)

Animal Husbandry Section

LAB/BRANCH
Comparative Medicine Branch

SECTION
Veterinary Medicine

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.25	PROFESSIONAL: 0.2	OTHER: 0.05

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to determine the effect of dietary protein levels on lifespan of the hamster as related to the severity of nephritis. An attempt will also be made to correlate the degree of nephritis with hypertension.

Weanling male and female golden Syrian hamsters will be fed either 6, 12, 18 or 24 percent protein in a pelleted ration. The mean lifespan of each group will be calculated and survivors will be killed when 20% of a group remains. All hamsters will be necropsied and the kidneys will be examined histologically for nephritis. Other major organs will also be examined for lesions. Prior to necropsy of survivors, blood pressure will be determined by cannulation of the carotid artery.

PROJECT DESCRIPTION

METHODS EMPLOYED: 180 (90 male and 90 female) weanling golden Syrian hamsters will be divided into 4 groups, each group receiving either 6, 12, 18 or 24 percent dietary protein *ad libitum* in a pelleted ration. The feed will be formulated every 90 days and analyzed for protein, fat, carbohydrates, lead, arsenic, cadmium, mercury, aflatoxins and antibiotics.

The animals will be housed individually in plastic cages (8' x 5' x 5' high) with hardwood bedding. Water will be supplied *ad libitum* in plastic bottles with stainless steel sipper tubes. Feed will be supplied in overhead cage top feeders. Weight gains will be recorded weekly on each animal until body weight plateaus, or does not increase more than 1% of the total body weight. Room temperature will be controlled (70°F ± 2° plus R.H. 50%) and lighting will be on a 12-hour light-dark cycle.

Complete necropsies will be performed on all dead or moribund animals, and histopathology will be done on a complete set of tissues. Major organs will be weighed. Blood will be collected at necropsy for hematology and serum chemistry. Urine will also be collected at necropsy and examined with standard multi-dip stick tests. Specific gravity and microscopic examination of sediment will also be performed.

The duration of survival of each group will be recorded and the differences compared by statistical methods. When 20% of a group remains, the survivors will be anesthetized with ketamine hydrochloride and halothane and blood pressure will be recorded after cannulating the carotid artery. This will be followed by complete necropsies, as above.

MAJOR FINDINGS AND PROPOSED COURSE: This project is due to commence shortly. The results of this project are to be published.

SIGNIFICANCE TO BIOMEDICAL RESEARCH OF THE PROGRAM OF THE INSTITUTE: NIEHS is increasing chronic toxicologic studies. It is imperative that research animals on these studies reach their maximum lifespan. Therefore, factors that increase or decrease mean lifespan in research animals is essential data. Hamsters are traditionally fed diets formulated for rats or mice, and which contain approximately 20% protein. Reducing the level of protein will delay the onset of nephritis in aging rats, but this has not been determined in the hamster. Hamsters on a lifetime carcinogenesis study die at a mean age of 56 weeks. Fifty percent die between 42-64 weeks, and fewer than 20% survive to 18 months. The reported lifespan of the hamster is 606-730 days. These animals are fed NIH-31, a diet containing 18-20 percent protein. This level may be too high for lifetime studies in the hamster.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30082-02 CMB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Comparison of Hamster Growth Associated with Hoarding and the Inability to Hoard Feed

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. B. Feldman	Veterinary Medical Officer	CMB NIEHS

COOPERATING UNITS (if any)
Animal Husbandry Section

LAB/BRANCH
Comparative Medicine Branch

SECTION
Veterinary Medicine

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is to compare feed consumption and weight gain in golden Syrian hamsters fed through overhead cage feeders versus feed placed directly into the cage (ability to hoard). The study will continue for 6 months, after which mean body weights of experimental groups will be compared. A difference of 20% in body weight will be considered statistically significant.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The animals will be divided into 2 groups. Group 1 will receive feed *ad limitum* through the feeder cage top; group 2 will have pelleted feed put directly onto the cage floor. Water will be supplied *ad libitum*. Feed consumption and animal body weights will be calculated weekly. At the end of 6 months, both groups will be compared for differences in mean body weight. A difference of 20 grams will be considered statistically significant (20% of body weight).

MAJOR FINDINGS AND PROPOSED COURSE: Results of this experiment revealed no significant differences in body weight between hamsters fed with overhead feeders versus those supplied with food in the cage. The experiment has been terminated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Hamsters are being used at NIEHS in increasing numbers for chronic studies. It is important that these animals reach both their maximum growth and life span. As long as hamsters thrive with overhead feeders, this method will be maintained, especially for those studies where feed consumption has to be measured.

[illegible]

ENVIRONMENTAL BIOLOGY BRANCH

ENVIRONMENTAL BIOLOGY BRANCH
Summary Statement

The Environmental Biology Branch assists and collaborates with NIEHS laboratories and research programs in the fields of inhalation toxicology, pathology, electron microscopy and histology, plans, conducts and collaborates in basic and applied studies in these fields, and plans, conducts and coordinates toxicologic bioassays on selected environmental chemicals.

The comparative toxicity of halogenated polyaromatic hydrocarbons, a principle focus of activity for several years, continued with specific chlorinated dibenzofuran and brominated naphthalene isomers being of current interest. The isomers to be studied are first determined based on predicted toxicologic properties and synthesized by the Environmental Chemistry Branch. The toxicity data further supports the previously formed hypothesis that toxicity is dependent on degree and specific location of halogenation of the aromatic ring and that the toxicity observed is a general syndrome suggesting a common mechanism of action.

Toxicopathology studies of polybrominated biphenyl (PBB) focused on the chronic effect of a six-month exposure in rats and mice and the effectiveness of charcoal, cholestyramine, and caloric restriction in reducing the body burden of PBB. In the latter studies no method was found to significantly enhance PBB excretion.

In collaboration with the Laboratory of Behavioral and Neurological Toxicology, a series of experiments were initiated with Kepone in rats to establish the dose range in which fertility, reproduction or neurobehavioral effects are observed. Neurobehavioral effects were observed at doses below those which affected fertility and reproduction. Further experiments are in progress to more precisely characterize these toxic effects, determine their duration, and to determine if progeny of Kepone exposed females are at risk.

Several major long-term projects progressed according to schedule:

1. The three-species vinyl chloride study which seeks to assess the functions of age and duration of exposure upon carcinogenic response is currently undergoing pathology evaluation;

2. The rat inhalation study which seeks to compare the fibrogenic and neoplastic response of chrysotile asbestos that varies as to fiber length and of a fibrous glass is in the sixteenth month of a two-year study. Early results clearly indicate a difference in severity of lung fibrosis dependent upon the length of the chrysotile fiber; and

3. The oral asbestos studies in the hamster are currently in the pathology evaluation phase; rat studies are in their fifteenth

month with minimal mortality observed to date. Chrysotile, amosite, and crocidolite asbestos and tremolite are being evaluated through contract.

Responsibility for the Environmental Teratology Information Center (ETIC) was transferred to the Branch late in FY 78. Since July, 1975, ETIC has built a computer file of approximately 15,000 records and assembled a document library that contains every paper indexed. Each citation has been indexed with all bibliographic information, common and taxonomic name of test object, and Chemical Abstract Service (CAS) Registry Number. Titles may be searched using key words from the title.

The chemical Agent Registry has been updated to include chemical name fragments and Wiswesser Linear Notations (WLN). As a result, chemicals in the ETIC Agent Registry may now be searched by synonym; CAS number; fragment as part of a chemical name; molecular formula; complete structure or fragment structure using Wiswesser Linear Notation codes; structure or structural fragment by diagram; and functional or major chemical components by name (e.g., esters, ethers, epoxides, etc.).

The ETIC file is now one of the subfiles of the National Library of Medicine's TOXLINE (Toxicology Information Online) and is available through a nationwide NLM network of centers at more than 900 universities, medical schools, hospitals, Government agencies, and commercial organizations as well as a number of foreign centers.

The ETIC file is also one of the data bases provided by RECON (Remote Console) a remote-access computerized storage and retrieval system located at Oak Ridge National Laboratories and available to all Department of Energy divisions and contractors.

The Chemical Agent Registry has been made a part of the Environmental Protection Agency/National Institutes of Health Substructure Search System and thereby offers still another search capability.

An average of 33 inquiries a month requesting information about over 100 agents and almost every test object are received and processed directly by the ETIC staff. There is no way to determine the number of requests processed through TOXLINE and RECON since they do not monitor searches on those systems.

It is proposed that ETIC will continue to collect and process papers from current and previous years and provide regular updates of the ETIC/TOXLINE subfile and the ETIC/RECON data base. We hope to be able to add author abstracts to the file. The author's address is one new field to be added and is already a part of a number of the other files on TOXLINE. You will be able to identify experts in a particular area by searching with zip codes. Based upon the needs and requirements shown through reviewing search requests, other new files may also be added. Publications of special search subjects will be prepared and made available upon request. A bibliographic listing will be prepared and updated on a regular basis. Primary effort will go into the next major undertaking of ETIC. They will begin the extraction of more detailed information from each reference. This information will then be entered into a Teratology Data Bank.

The Comparative Pathology Section maintains a centralized NIEHS service facility for electron microscopy and histology, provides a diagnostic service for lesions observed in laboratory animals, provides a hematology and clinical chemistry service, and plans, conducts and collaborates with NIEHS research programs in research activities utilizing various techniques of experimental pathology.

The Section supported the disease surveillance program of the Comparative Medicine Branch by conducting necropsies and histopathologic interpretation of 843 cases during the year. It also provided histologic, EM or pathology support to 25 projects within six intramural research laboratories.

The program of the Inhalation Toxicology Section is composed of three parts: (1) studies are conducted of compounds to which toxicologically significant exposure would be expected to be primarily by inhalation. Research is focused on expressions of toxicity at the levels of tissues, organs, and organ systems. Emphasis is placed on interactions among the cardiovascular system, the lungs and kidneys. (2) A principle effort of the Section is directed toward the advancement of inhalation technology. (3) Support is provided to intramural scientists outside the Section in the form of consultation, and collaboration, where appropriate, in matters of inhalation toxicology. The Section also allocates up to 50% of its small animal inhalation exposure chamber capacity to studies originating outside the Section as a service.

The small animal inhalation facility is now operational under machine control. Data acquisition and chamber control are by means of an advanced electronic calculator rather than a minicomputer which, although somewhat slower and less versatile than a "mini", serves the job reasonably well. The system controls 6 pairs of chambers, but is designed to accomodate up to 9. As more chemical analytical instruments are acquired, more pairs of chambers may be operated, or multiple compounds may be run in the same chamber(s). The compounds of interest in the chamber are monitored with infrared gas analyzers whenever possible, but provision has been made for the use of gas chromatographs as well. Data acquisition and control are more efficient when the infrared analyzers are used.

Complete exposure data summaries are provided the investigator both during the exposures and at the conclusion of each experiment. This includes records of the variables monitored that are relevant to the exposure such as concentration of the compound of interest, and chamber temperature and humidity. Furthermore, calibration data, quality control summaries, and estimates of the error in achieving the target profile are provided. These summaries take the form of printed tables and graphic summary of the exposure profile, generated by a peripheral plotter that is of a quality suitable for reproduction.

The facility is designed for exposure to organic vapors, cylinder gases, and oxides of nitrogen. We are not equipped for ozone, or solid or liquid aerosols.

We have allotted one-half of our total exposure capacity to projects originating outside the Inhalation Toxicology Section. Exposures of laboratory animals to benzene, carbon disulfide, and m-xylene have been performed for scientists in the Intramural Program and the Environmental Biology Branch.

The subject of the detection of leaks (into the room) from chambers containing particulates arose at a conference held during the past year. The suggestion was made than an inert gas like SF_6 might be added to the chamber supply air to facilitate rapid leak detection. Another potential use for such a gas in tracer concentrations might be for the indirect monitoring of otherwise difficult to monitor gases like NO_2. The SF_6 could be mixed in a constant ratio with the NO_2, for example, and the SF_6 concentration followed as an index of the NO_2 concentration. Studies are now underway to verify the concept. The follow-up to this could include longer term, low levels studies of SF_6 toxicity for the purpose of clearing the way for its further consideration as an inert chamber air additive.

The Section research program is divided into five parts. They are studies of environmental contaminants on (1) the electromechanical activity of isolated, perfused whole hearts and other myocardial preparations in vitro, (2) the composition and function of pulmonary lavage and its constituents, (3) pulmonary function in small animals and (4) a new study to test the hypothesis that exposure of laboratory animals to NO_2 during concurrent administration of heterocyclic amines leads to the formation in vivo of nitrosamines that result in tumor formation. A fifth project serves as an umbrella for miscellaneous, related studies and is called Toxicology of Environmental Chemicals.

Isolated perfused rabbit hearts and other heart preparations are being used for the purpose of evaluating the effects of CS_2 on myocardial function. Under some circumstances a marked rise in end diastolic pressure has occurred during or after exposure of the hearts to 0.1-1.0 ml/l of CS_2.

A negative inotropic effect is also observed, regardless of whether or not the contracture is observed. The contracture has not been conclusively ascertained not to be artifactual. The negative inotropic effect has been shown to be accompanied by a rise in oxygen consumption. Studies are being conducted to verify that the contractures that have been observed represent a genuine toxic response of the heart to the CS_2, and, if so, through what mechanism. The matter of the inverse relationship between potential work and oxygen consumption is being investigated using isolated mitochondria.

Pulmonary lavage is easily obtainable from rabbits, and studies of its composition and properties of its constituents are being investigated as early indicators of toxic injury to the lung. Preliminary studies suggest that alterations in lectin binding by alveolar macrophages may be associated with alterations in extracellular enzyme activities rather than of intracellular activities as a consequence of the inhalation exposure of the host rabbits to morpholine. Because of the difficulty of exposing large numbers of rabbits within our facility, correlative studies are being undertaken using cultured macrophages as a substitute for whole animals.

Functional changes in respiratory physiology are slowly gaining recognition as valid indicators of certain types of toxic injury in laboratory animals, e.g. small airway obstructive injury. Pulmonary function has been evaluated in rats exposed to morpholine by inhalation and evidence of small airway obstruction has been detected in the absence of definitive histopathological changes. This observation serves to emphasize that pulmonary function testing should be considered a potentially useful tool in the assessment of toxic injury to the lung. Similar studies have been conducted in rats treated with either paraquat or diquat, well-known pulmonary toxicants. Pulmonary function testing has served to distinguish between the two chemicals. Functional impairment was far more severe with paraquat intoxication than with diquat. Few investigators have paid much attention to the matter of the effects of anesthesia on the outcome of pulmonary function tests in small laboratory animals. Our battery of tests is being performed on rats anesthetized with a large number of different anesthetics and anesthetic combinations for the purpose of aiding in the selection of agents most suitable for use in laboratory studies.

The observation that nitrosation of heterocyclic amines will take place at a rapid rate and with a high yield in alkaline or neutral media in the presence of NO_2 has given rise to the idea that NO_2 could be linked to carcinogenesis through heterocyclic amines. Rats, mice and hamsters have been exposed to 1-2 ppm of NO_2 while receiving morpholine in the drinking water. Exposures took place 5 days per week for 30 weeks. The animals will be held for at least one year before histopathological examination. Preparations for a related study are underway in which the animals will be exposed to 10 ppm of NO_2 while receiving 2,6-dimethylmorpholine in the drinking water. The exposures will be conducted 5 da/wk for 2 yr. Both nitrosomorpholine and nitroso-2,6- dimethylmorpholine are potent animal carcinogens.

Morpholine is excreted in the urine and the bile. A series of experiments is underway to characterize the renal handling of morpholine in the rabbit, the species whose renal function was shown to be the most sensitive among the rat, guinea pig, and rabbit, using the kidney slice technic. Evidence acquired so far does not support the original working hypothesis that morpholine is excreted by the organic base tubular transport system.

Prior to the construction of a computerized inhalation chamber monitoring and control system, the faithful generation of time-varying exposure profiles repeatedly was technically, virtually impossible. Consequently, the overwhelming majority of inhalation studies performed in the past has been done using profiles in which the chamber concentration has been brought up to some level, held there for a specified period, and then brought down. We are able in our facility to reproduce rapidly changing, time-varying exposure profiles under machine control. In order to assess the significance on the outcome of experiments in inhalation toxicity of the shape of the exposure profile, we have performed a series of exposures of rats to CCl_4 at constant concentration times time, but in different profile. The assessment of toxic injury is based on serum sorbitol dehydrogenase activity and histopathological examination of the liver.

A commitment of Branch scientists is to the recently established National Toxicology Program. In addition to laboratory research, scientists regularly participate in experimental design and subsequent interpretation of lifetime rodent bioassays. Contract studies which are researching the increased sensitivity of laboratory animals to environmental chemicals during in utero and post natal development are also an NTP program.

Expansion of the toxicology capabilities of the Branch was realized through the successful recruitment of a renal toxicologist and an immunologist. These scientists are to develop and validate reliable and sensitive protocols for assessing toxic effects that can be utilized in the test phases of the NTP.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80020-07 EBB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Changes in Mammalian Pulmonary Function Produced by Inhaled Environmental Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H.F. Lam	Visiting Fellow	EBB NIEHS
OTHER:	E.W. Van Stee	Physiologist	EB NIEHS
	R.A. Sloane	Bio Lab Technician	EBB NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Environmental Biology Branch

SECTION
Inhalation Toxicology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.1	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Changes in pulmonary function resulting from inhalation of airborne pollutants are being estimated using a whole body pressure plethysmograph constructed for use with small animals. Both acute and chronic exposure to oxidants, singly and in combination with other compounds result in functional changes. Changes in pulmonary mechanics (e.g. resistance, compliance), spirometry (e.g. lung volumes, frequency of breathing), heart rate, and pulmonary morphology will be estimated.

PROJECT DESCRIPTION

METHODS EMPLOYED: We have established the methods to assess the changes in pulmonary function induced by environmental toxicants. Our methods include single breath diffusing capacity for carbon monoxide in the lung (D_{LCO}), neon dilution to measure lung volumes such as residual volume (RV), functional residual capacity (FRC), and total lung capacity (TLC), and methods to measure static compliance and resistance in small laboratory animals.

MAJOR FINDINGS AND PROPOSED COURSE: D_{LCO} and lung volumes of anesthetized male hamsters, rats, guinea pigs and rabbits, whose weights varied from 40 to 3500g, have been measured (1). A high correlation has been established between body weight and both the diffusing capacity and the lung volumes. In rats, D_{LCO} did not change with different body positions, at airway pressures of 10 or 20 cmH_2O, from FRC or RV or following hyperventilation. Morpholine was selected as the first compound to evaluate the sensitivity of our method to detect the damage in the lung. Pulmonary function testing was often more sensitive than light microscopical examination for detecting lung effects of inhaled toxicants. We have found that acute (2000 ppm) and subacute (450 ppm) exposure of morpholine to male rats induced changes in pulmonary function which suggested obstructive lung defect (2).

Paraquat, a chemical responsible for many deaths from accidental or intentional poisoning, has been the subject of human and experimental studies on its toxic manifestation. Since the time-related effect of paraquat and diquat on lung function of animals has not been studied, an investigation was made to clarify the physiological or clinical picture of intoxication. Rats injected with paraquat (27 mg/kg I.P. or 0.5 mg/kg I.T.) had a progressive decrease in D_{LCO}, lung volumes and compliance over the measurement period of 72 hours. Diquat has little effect on lung function. The results obtained resemble in many ways the effect reported in human intoxication (3,4).

Interest in lung function during general anesthesia arises because the incidence of pulmonary problems in clinical practice is high and because the lung is the site of uptake and elimination of volatile and gaseous anesthetics. Surprisingly, many investigators have paid little attention to anesthesia in their experiments even though it is generally known that anesthetic agents interfere with pulmonary gas exchange and could cause an alteration in pulmonary mechanics. The anesthetic used may have important interaction with some experimental parameters and this could alter the results considerably. An investigation is being carried out to examine the effect on the respiration rate, lung volumes, D_{LCO} and compliance of animals under ten different anesthetic treatments. Impaired pulmonary gas exchange can be detected by a decrease in D_{LCO} and alveolar volume (FA), the change in ventilation distribution by a decrease in VA, FRC and other lung volumes and the constriction of alveolar ducts or respiratory bronchioles by the alteration in lung compliance. A comparison will be made between the various anesthetics in their ability to impair lung function. It is hoped that results from this experiment will make investigators more aware of the problem and help them in their choice of anesthetic.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Changes in pulmonary function of animals after exposure to inhaled environmental chemicals are known, in many cases, to be associated with cellular damage and morpholine vapors (250-450 ppm) for many hours has been demonstrated to induce functional changes in small laboratory animals while histopathological observations were still indicating reversible small changes or no change. The development of tests which will given an accurate measurement of early airway obstruction before structural changes could be detected morphologically, is an important step forward. Small airway obstruction is an "early warning" signal before the development of normally irreversible destructive lung lesions and pulmonary function tests can be a valuable tool in the screening of chemicals which could induce pulmonary abnormalities.

PUBLICATIONS

Takezawa, J., Miller, J.M., and O'Neil, J.J.: Lung volume and the single breath diffusing capacity for carbon monoxide measured in small laboratory mammals. Am. Rev. Resp. Dis. 117: 405, 1978.

Takezawa, J., and Lam, H.F.: Toxic effect of morpholine on rats lung. Fed. Proc. 37: 247, 1978.

Lam, H.F., Takezawa, J., and Van Stee, E.W.: The effects of paraquat and diquat on lung function measurements in rats. Am. Rev. Resp. Dis. 119: 327, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES30038-02 EBB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

An Evaluation of Contribution of Chlorinated Dibenzodioxins to Hexachlorobenzene Toxicity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.A. Goldstein	Pharmacologist	EBB NIEHS
OTHER:	P. Linko	Chemist	EBB NIEHS

COOPERATING UNITS (if any)

T. Scotti, EPA

LAB/BRANCH
Environmental Biology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.1	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study was initiated to study the effects of acute and chronic hexachlorobenzene (HCB) on drug metabolism, porphyria lung and liver histology and clinical signs are not related to chlorodibenzodioxin contamination. Work is continuing to identify the cytochromes induced by HCB and classify HCB as an inducer of hepatic enzymes.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: The effects of HCB (porphyria, induction of liver mixed function oxidases (MFOs), histological changes and neurological signs) appear to be due to HCB, not its contaminants. However, its acute effects on MFOs indicate that it shares some of the properties of dibenzo-p-dioxins. The type of cytochromes induced and substrate pattern is being investigated to determine whether HCB does indeed have some of the inductive properties of dioxins. Work may be extended to other representative chlorinated benzenes to determine whether HCB is unique in this respect. Work on this project is continuing.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: HCB is a widespread environmental contaminant. HCB resembles the more toxic dioxins in some of its effects. These compounds are quite different structurally. It is important to know whether the HCB and dioxins operate through the same mechanism and to be able to predict the structure-activity relationship since toxicity and induction appear to be related.

PUBLICATIONS

Goldstein, J.A., Friesen, M., Scotti, T.M., Hickman, P., Hass, J.R., and Bergman, H. Assessment of the Contribution of Chlorinated Dibenzo-p-dioxins and Dibenzofurans to Hexachlorobenzene-Induced Toxicity, Porphyria, Changes in Mixed Function Oxygenases and Histopathological Changes. Tox. Appl. Pharmacol., 46,633-649, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30042-04 EBB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of Environmental Contaminants on Cardiac Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. W. Van Stee	Physiologist	EBB NIEHS
	M. P. Moorman	Biomedical Engineer	EBB NIEHS
	R. Sloane	Biological Laboratory Technician	EBB NIEHS

COOPERATING UNITS (if any)

Pathology Section, EBB

LAB/BRANCH
Environmental Biology Branch

SECTION
Inhalation Toxicology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.40	PROFESSIONAL: 0.25	OTHER: 0.15

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is directed toward the detection of functional changes in the cardiovascular system associated with compounds of environmental significance to which toxicologically significant exposure would be expected by inhalation.

Carbon disulfide-present in perfusate at concentrations as low as 100 ppm (v/v) has caused contracture in isolated, perfused rabbit hearts under certain experimental conditions. A series of experiments is underway to verify and characterize this phenomenon more fully and, if possible, determine the physiological mechanism of action.

PROJECT DESCRIPTION

METHODS EMPLOYED: The effects of environmental chemicals on the heart are studied using isolated, perfused (Langedorff) rabbit hearts and other isolated heart muscle preparations. Chemicals used are carbon disulfide (CS_2), $^{45}CaCl_2$, and ryanodine.

MAJOR FINDINGS AND PROPOSED COURSE: Exposure of isolated, rabbit hearts perfused with Dulbecco's modified Eagle's medium at 30°C and equilibrated with 95% O_2-5% CO_2, to CS_2 has resulted in contracture at concentrations from 100-1000 ppm (v/v). The hearts are taken from rabbits previously depleted of endogenous catecholamines with 6-OH-dopamine. The Bundle of His is transected and the hearts paced from a site on the right ventricular septum. We conclude at this point that the contracture is independent of endogenous catecholamines and the His-Purkinje system. A series of experiments is scheduled to investigate this phenomenon further. Experiment 1: Evaluation of CS_2 effects on the staircase phenomena (Woodworth and Bowditch). Experiment 2: $^{45}Ca^{++}$ uptake and washout studies. Experiment 3: Studies of the effects of CS_2 on the affinity of isolated fragments of sarcoplasmic reticulum (relaxing factor) for calcium. Experiments 4 and 5: Effects of CS_2 on mitochondrial respiration and calcium flux. Experiment 6: Effects of CS_2 on the superprecipitation of the myocardial actomyosin complex. Experiment 7: Electrophysiological studies will be performed on both the isolated whole hearts and superfused papillary muscles. Other possible approaches: Depending on the outcome of the staircase experiments, further studies may be done in hearts from animals treated with ryanidine, an insecticidal chemical that selectively destroys the connection between the t-tubules and sarcoplasmic reticulum. Further studies also could include consideration of possible interactions of CS_2, calcium, and the glycocalyx (baseman layer external to unit membrane of the myocardial sarcolemma). Such studies would be likely to require the use of various organic and ionic calcium antagonists.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Carbon disulfide has been selected as one of the initial chemicals for evaluation in the National Toxicology Program.

PUBLICATIONS

Van Stee, E.W. (1979) Myocardial Toxicity, Proc. Symp. Sci. Basis of Toxicity Assessment, Elsevier, Amsterdam (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30044-03-EBB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Toxicology of Environmental Chemicals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. W. Van Stee	Physiologist	EBB NIEHS
	E. G. Tombropoulos	Research Chemist	EBB NIEHS
OTHER:	M. P. Moorman	Biomedical Engineer	EBB NIEHS
	F. W. Harrington	Biological Laboratory Technician	EBB NIEHS
	P.C. Wynns	Biological Aid	EBB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Environmental Biology Branch

SECTION
Inhalation Toxicology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.85	PROFESSIONAL: 0.1	OTHER: 0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Morpholine is excreted in the urine and bile following exposure by any route. The chemical might be expected to be excreted via the renal tubular base transport system, but evidence indicates that this may not be so. Furthermore, although its solubility may be controlled through regulation of urinary pH, its rate of elimination in the urine is not. Studies are continuing to elucidate the renal disposition of this chemical. Preliminary studies indicate that time-varying concentration profiles affect expressions of inhalation toxicity. Rats exposed to a variety of profiles with equal concentration times time, exhibit different degrees of chemical injury to the liver by carbon tetrachloride.

PROJECT DESCRIPTION

METHODS EMPLOYED: Rabbit renal cortical slices are incubated in Dulbecco's modified Eagle's medium or Daniel's Minimum Essential Medium. Isolated kidneys are perfused in an apparatus of standard general design. Clearances are determined by conventional methods. Morpholine labeled uniformly with ^{14}C, TEA labeled with ^{3}H, and PAH labeled with ^{14}C are used in the studies. Oxygen consumption of tissues is determined using an oxygraph. Cold chemicals of particular interest used are morpholine, 2-aminoethoxyethanol, diethanolamine, 2,6-dimethylmorpholine, TEA and PAH. Male Fisher rats, 100-150 g are exposed to air or various time-varying concentration profiles of CCl_4 that all have a concentration times time equal to 1500 ppm-hr. Serum sorbitol dehydrogenase activities and histopathological evaluation of the livers are used as indexes of hepatoxicity.

MAJOR FINDINGS AND PROPOSED COURSE: Rabbits exposed to morpholine by inhalation appear to excrete the compound largely via the kidneys early (1st 24 hours postexposure). Our original working hypothesis was that morpholine was excreted by the kidneys via the organic base tubular transport system. We have since been unable to establish a single piece of evidence to support the hypothesis. Experiment 1: Morpholine at concentrations of 10^{-3} M or less does not impair oxygen consumption of rabbit renal cortical slices in vitro. Experiment 2: Since the pH of the medium affects the degree of hydrogen ion association of morpholine, slices were studied in the oxygraph at different pH. No difference in oxygen consumption was observed from pH 7.4-8.3. Experiment 3: pH affected the distribution of morpholine between aqueous incubation medium and heptane. Morpholine was preferentially distributed to the heptane by increasing pH and preferentially distributed to the medium by decreasing pH. Experiment 4: Morpholine at concentrations of 5×10^{-3} M did not affect renal cortical slice uptake of TEA. Experiment 5: The rate of decline of blood levels of morpholine over 48 hr after bolus injection IV was unaffected by making the urine alkaline by chronic pretreatment with $NaHCO_3$, or by acidifying by pretreatment with either ascorbic acid or NH_4Cl. Future experiments planned in this series are as follows. Experiment 6: Repeat of earlier experiment that yielded inconclusive results in which renal cortical slices are incubated with morpholine in media equilibrated with 95%-5% O_2 and CO_2 or 95%-5% N_2-CO_2 for the purpose of making a quantitative distinction between active uptake and nonspecific binding. Experiment 7: Verification of organ distribution of morpholine determined by GC using very few animals and radiolabeled morpholine. Experiment 8: Temporal profile of excretion of radioactivity in urine and bile following radiolabeled morpholine injection. Experiment 9: Analysis of radioactive excretory products for possible metabolites. Experiment 10: Quantification or rate of excretion of morpholine via lungs as $^{14}CO_2$ and ^{14}C-morpholine. Experiment 11: Measurement of the ratio of renal clearances of morpholine to inulin in anesthetized rabbits. Experiment 12: Preliminary studies for the purpose of assessing the potential utility of the isolated, perfused kidney as a model in our laboratory.

Preliminary studies indicate that differences exist among the treatment groups. Since each group is exposed to the same C x T, the variations in degree of toxic injury to the liver must be attributable to the exposure profile. Animal supply problems have made it necessary to halt the present series of experiments. A new set will be conducted on animals with a uniform genetic background as soon as the supply of animals is reinstated. The ultimate objective will be to determine what features of the exposure profile contribute to the expression of toxicity and to what degree. Variables like C x T, number of leading edges, maximum concentration, etc, will be analyzed using a multiple regression approach.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Little is known, in general, regarding the specific renal pharmacology of heterocyclic amines. The amines are of general toxicological significance because of their potential for conversion to nitrosamines known to be significant animal carcinogens. A comprehensive approach to nitrosamine toxicology requires the inclusion of studies of the precursors, when such information is not otherwise available. Only through an understanding of the contributions of the individual parts of a hypothetical scheme can one hope to appreciate the significance of the in vivo interactions that may lead to the final endpoint which, in this case, is the expression of nitrosamine toxicity in an animal model.

SUMMARY: Morpholine and related amines are subject to conversion to the corresponding nitrosamines by nitrite at acid pH and by NO_2 (N_2O_4) at neutral and alkaline pH. The nitrosamines may be significant animal carcinogens. Thus, the potential exists for chemical interactions that would lead to the formation in vivo of nitrosamines. A complete understanding of the whole process requires that information be acquired not only in regard to the proximate toxicant (nitrosamine) but also the precursors (e.g. heterocyclic amines). The kidney appears to be a significant portal of excretion for heterocyclic amines and, as such, a goal of this research is the elucidation of the renal pharmacology of the amines using morpholine as the principal model compound. Computerized inhalation facility operation has removed a barrier to the reliable reproduction of time-varying exposure profiles in the inhalation toxicology laboratory. Experiments in inhalation toxicology traditionally have centered around non-time-varying exposure profiles in which chamber concentrations were rapidly raised to some predetermined point, held there for a specified period, and then reduced to zero as rapidly as possible. This may not always be the best model for the system under study. Our preliminary results indicate that when the traditional approach does not accurately represent the system under investigation, the inferences may be erroneous.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30045-03 EBB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Development of an Automatic Small Animal Inhalation Facility

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. P. Moorman	Biomedical Engineer	EBB NIEHS
	E. W. Van Stee	Physiologist	EBB NIEHS
OTHER:	F. Harrington	Biological Laboratory Technician	EBB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology Branch

SECTION
Inhalation Toxicology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	.5	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project involves the design and implementation of a microcomputer based system to monitor and control gas concentrations in a small animal inhalation exposure facility. Included in this effort are the development of hardware, software and operational procedures necessary for the complete utilization of the system. Sulfur hexafluoride is being evaluated for use as a tracer gas in the monitoring of chamber additives and for chamber leak detection.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Data acquisition and feedback control theory have been used to develop a microcomputer based sampled data control system capable of regulating 9 chambers on a time multiplex basis.

MAJOR FINDINGS AND PROPOSED COURSE: Since this system must regulate gases generated from compounds with different physical properties, it has been necessary to design a control system capable of measuring certain characteristics of each generating system and adapting the control equations to optimize responses for each particular compound.

Because equipment calibration is a significant factor in system performance statistical procedures have been implemented to evaluate daily calibrations and long term system performance.

In order to better define the exposure environment, temperature and humidity are monitored and animal excrement actively removed during the course of the exposure.

Further refinements in the computer management of calibration procedures and in the construction of the data conversion equation are necessary to bring this system to its full potential.

The realization of maximum usefulness of this project requires detailed documentation of system hardware, software, and operating procedures. System performance must be documented through use.

The addition of an inert tracer gas to the chamber ventilation mixture would serve two purposes. The first is the monitoring of otherwise difficult to detect gases being added to the chamber, and the other is leak detection from the chambers into the extramural environment.

SF_6 has been mixed in constant ratio with NO_2 in compressed gas cylinders. Studies are underway in which correlations are being established between the concentrations of SF_6 and NO_2 in the chambers, with the goal in mind of using the SF_6 concentration as an index of the NO_2 concentration. The reason for using this approach is that the monitoring of NO_2 requires the use of a discrete sampling device in conjunction with special calibration equipment. SF_6 may be monitored using the infrared gas analyzers that are the main analytical instruments preferred for use in the automated facility. NO_2 pulmonary toxicity studies are being conducted on rats in a 2 x 2 layout in which matched groups are exposed to either air, NO_2, SF_6, or NO_2+SF_6 in order to provide preliminary assessment of any contribution that the SF_6 might make to the NO_2 toxicity. The results of many studies on SF_6 toxicity done elsewhere suggest that its biological inertness will be confirmed in our studies.

Long-term, low level (tracer concentrations) studies of SF_6 may be conducted in the future in preparation for clearing the way for the addition of SF_6 to chamber supply air for the purpose of leak detection. This would be especially useful in particulate exposures, for example, in which sampling procedures take a sufficient length of time that a hazard to personnel may be present before the chamber room air samples have been collected and processed. SF_6 would appear in the room in the case of a leak and would be detectable immediately. If the decision were made to add SF_6 to the chamber air supply manifold rather than to individual chambers and thus deliver it to all chambers indiscriminately, a single leak detector could be in operation in the room full time. In the event that SF_6 were detected, procedures could be initiated quickly for location of the source of the leak and identification of potential hazard according to the source.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

1. Machine control of inhalation chambers can produce an accurately controlled and documented exposure resulting in reduced technical error.

2. Statistical evaluation of periodic calibrations can quantify monitoring accuracy and identify many calibration errors.

3. Documentation of the exposure conditions and profiles can be summarized in a compact and meaningful format eliminating the need for hand analysis of chamber output data.

4. More complicated exposures such as time varying concentrations of multiple compounds are possible with machine control more than human operators.

5. SF_6 added to NO_2 may improve the efficiency of inhalation chamber monitoring and control of chamber NO_2 concentrations.

6. SF_6 used in tracer concentrations for chamber leak detection would improve the efficiency of leak detection from chambers into the chamber room environment and thus contribute to improved safety of chamber operation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30073-02 EBB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Efforts to Enhance the Elimination of 2,3,7,8-Tetrachloro-dibenzo-p-dioxin from the Body of Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E.E. McConnell	Veterinary Pathologist	EBB NIEHS
OTHER:	H.B. Matthews	Research Chemist	LPK NIEHS
	J.A. Moore	Supervisory Veterinary Medical Officer	EBB NIEHS
	M.W. Harris	Biological Laboratory Technician	EBB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology Branch

SECTION
Comparative Pathology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The object of this study was to determine if cholestyrimine, mineral oil or charcoal had any effect on enhancing the rate of elimination of 2,3,7,8-Tetrochlorodibenzo-p-dioxin (TCDD) from the body of rats.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Fischer strain female rats were given diets containing either cholestyrimine, mineral oil or charcoal. (^{14}C) TCDD was injected intraperitoneally either 7 days before or 7 days after the rats were placed on the diets. Radioactivity was monitored in the feces and urine for 14 days after TCDD exposure. Liver and fat obtained at necropsy were also evaluated for radioactivity.

MAJOR FINDINGS AND PROPOSED COURSE: Cholestyrimine and mineral oil did not enhance elimination of TCDD from the body of rats. However, charcoal dramatically enhanced elimination and reduced body burden if used prior to TCDD exposure but had little effect if given 7 days post exposure. This project will be completed this FY.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Chlorinated dibenzodioxins are extremely toxic compounds which may find their way into the environment *via* contamination of several useful chemicals. Significant human exposures have occurred in many areas of the world. Since these exposures are usually of a chronic (prolonged) nature it would be extremely useful to find a therapeutic compound which would both reduce absorption and enhance elimination of these chemicals from the body.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30075-02 EBB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Comparative Toxicity of Technical vs Pure Pentachlorophenol in Dairy Cattle

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E.E. McConnell	Veterinary Pathologist	EBB NIEHS
	C.E. Parker	Research Chemist	ECB NIEHS
OTHER:	J.A. Moore	Senior Veterinary Medical Officer	EBB NIEHS
	B.N. Gupta	Veterinary Pathologist	EBB NIEHS
	M.I. Luster	Research Chemist	ECB NIEHS
	J.A. Goldstein	Research Pharmacologist	EBB NIEHS
	A.H. Rakes	Animal Scientist	NCSU
	R.E. Wilson	Biological Laboratory Technician	EBB NIEHS
	F.A. Talley	Histology Technician	EBB NIEHS

COOPERATING UNITS (if any)

Animal Science Department, North Carolina State University, Raleigh, North Carolina

LAB/BRANCH
Environmental Biology Branch

SECTION
Comparative Pathology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.4	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this 160 day feeding study was to determine the effects of unwanted contaminants in technical pentachlorophenol (PCP) in dairy cattle by comparing them to pure PCP and non-exposed animals. Parameters studied included clinical examination, weight gain, hoof growth, hematology and clinical chemistry, gross and histopathology, electron microscopy, immunology, hepatic enzyme induction, and chemical analysis of tissues. Results show that technical PCP is significantly more toxic than pure PCP.

PROJECT DESCRIPTION

METHODS EMPLOYED: The objective of this study was to compare the effects of technical (t) vs analytical (a) grade PCP in cattle. Four groups of 3 female yearling Holstein calves were exposed for 160 days via feed to aPCP or tPCP and mixtures thereof. A fifth group served as unexposed controls. All treated animals received the same amount of PCP; 20 mg/kg/day for 42 days followed by 15 mg/kg/day. Fat biopsies for chemical analyses were obtained before, during (70 days) and at the end of the study. Liver samples were collected at the latter two times. Parameters studied included clinical signs, feed consumption, weight gain, hoof growth, hematology and clinical chemistry, urinalysis and immunology. At the termination of the experiment the animals were necropsied and representative tissues were collected for histopathology, electron microscopy, hepatic enzyme induction evaluation, immunology, and chemical analysis [see ECB (Parker) for details].

MAJOR FINDINGS AND PROPOSED COURSE: Results show that technical PCP is significantly more toxic than pure PCP. Major findings included a dose related decrease in body weight gain, decreased feed efficiency and progressive anemia in tPCP cattle while those exposed to aPCP were comparable to the untreated controls. Liver and lung weights were increased while the thymus was decreased. Marked villous hyperplasia of the urinary bladder was found in 2 of 3 animals exposed to the highest level of tPCP. There were minimal hepatic lesions. Immunological studies suggested a progressive dose related alteration in cell mediated immunity but no effect on humoral immunity. Liver mixed function oxidases were increased moderately by aPCP, but markedly by tPCP. This study indicates that the toxicity of PCP in cattle is primarily attributable to its contamination with toxic impurities. Project will be completed this FY.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM AT THE INSTITUTE: Pentachlorophenol is widely used as a wood preservative and fungistatic agent. In some cases it is contaminated with unwanted toxic chemicals such as dibenzo-p-dioxins. There have been clinical situations in which PCP treated wood has been incriminated as a etiologic factor in diseased dairy cattle in the north central United States. Because of this farm problem and the potential of contaminated food (beef) reaching humans, it is important to assess the toxicity of PCP in dairy cattle and to determine the importance of contaminants therein.

PUBLICATIONS

McConnell, E.E., Moore, J.A., Gupta, B.N., Rakes, A.H., Luster, M.I., Goldstein J.A., Haseman, J.K., and Parker, C.E. This chronic toxicity of technical and analytical pentachlorophenol in cattle. Tox. Appl. Pharmacol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30077-02 EBB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Dose Response to the Carcinogens, 1,2-Dimethylhydrazine (DMH) and Methylazoxymethanol (MAM) in Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. E. McConnell	Veterinary Pathologist	EBB NIEHS
OTHER:	J. A. Moore	Supervisory Veterinary Medical Officer	EBB NIEHS
	R.E. Wilson	Biological Laboratory Technician	EBB NIEHS

COOPERATING UNITS (if any)

J. Fawkes, ECB

LAB/BRANCH
Environmental Biology Branch

SECTION
Comparative Pathology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The "HEW Committee to Coordinate Toxicology and Other Related Programs" has designed a study to establish the biologic consequence from ingestion of asbestos. NIEHS has the responsibility for effecting these protocols. One aspect of the study is to determine if asbestos can act as a co-carcinogen, specifically in the gastrointestinal tract. 1,2-Dimethylhydrazine (DMH) and methylazoxymethanol (MAM) are known intestinal carcinogens, however, the dose required to produce a relatively low incidence (15 ± 5%) of colon cancer is not known. The objective of this study is to derive this information and in addition, to critically evaluate any neoplasm produced as to location, size, and morphology.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Five week old male and female Fischer (CDF) rats were given various amounts (30, 15, 7.5, 4, 1.0 and 0.2 mg/kg) of either DMH or MAM *via* gavage. They were dosed every other week for 8 weeks (5 doses). Body weight gain was monitored. The study was terminated at 12 to 14 months after the first dose. The incidence of neoplasia and latent period was recorded. Tumors were recorded as to their location, number, size and morphology.

MAJOR FINDINGS AND PROPOSED COURSE: There was a small but significant dose related decrease in weight gain in the 30 mg/kg animals (both compounds). The findings suggest that the dose of both compounds required to produce a 15-25% incidence of colon neoplasia is 15 mg/kg in female and 7.5 mg/kg in male rats. However, there were more tumors in other locations with MAM. For this reason, DMH was considered a better model for use in the asbestos study. Project has been completed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Colon cancer is the 2nd leading form of neoplasia in men and 3rd in women. Various studies have indicated that it is probably environmentally related. Suggestions have been made that asbestos may play a role in the causation. The development of a method which would yield a relatively low incidence would serve as a useful model for the study of this important disease.

PUBLICATIONS

McConnell, E.E., Wilson, R., and Moore, J.A. Dose response of 1,2-dimethylhydrazine and methylazoxymethanol acetate in the F344 rat. *J. Nat. Cancer Inst.* (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30084-02 EBB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Dose-response of Drug-metabolizing Enzymes to Halogenated Biphenyls and Napthalenes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.A. Goldstein	Pharmacologist	EBB NIEHS
OTHER:	P. Linko	Chemist	EBB NIEHS
	J.D. McKinney	Supervisory Research Chemist	EBB NIEHS

COOPERATING UNITS (if any)
B. Gupta, Comparative Pathology
L. Levy, ECB
J. Weaver, NCSU

LAB/BRANCH
Environmental Biology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.3	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The polybrominated biphenyls (PBBs) and polychlorinated biphenyls (PCBs) are inducers of hepatic mixed-function oxidases. The purpose of this study is to determine the structure-activity relationships of PCB and PBB isomers and other components of the commercial PBB and PCB mixtures, as inducers of cytochromes P-450 and P-450 and to relate these effects to toxicity.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Female rats were injected i.p. with various equimolar doses of PCB or PBB isomers, and PCB or PBB mixtures. Effects on liver and body weight, benzpyrene hydroxylase (AHH), aminopyrine N-demethylase, cytochrome P-450 and ethyl isocyanide ratios were compared. Liver samples were taken for residue analysis by neutron activation and porphyrin analysis.

MAJOR FINDINGS AND PROPOSED COURSE: A final report was prepareed of our work on the commercial PBB mixture involved in the Michigan poisoning (Firemaster BP-6) and of its major components, 2,4,5,2',4',5'-hexabromobiphenyl (HBB) and a brominated naphthalene (TBN) isomer believed to be representative of the naphthalene components of Firemaster. This report indicated that Firemaster is a mixed inducer, intermediate in its effects between pheonbarbital and 3-methylcholanthrene (MC). HBB resembles phenobarbital, and TBN is an MC type inducer but not potent enough to account for the mixed effects of the inducer. Additional work was done on an unsymmetrical PCB isomer, 2,3,5,3',4',5'-hexachlorobiphenyl (HCB), which was compared to its symmetrical analogs. 2,3,5,2',3',5'-HCB is a phenobarbital-type inducer; 3,4,5,3',4',5'-HCB an MC-type, but the unsymmetrical compound was essentially devoid of inductive activity.

A review on the "structure activity relationships and biomedical effects of halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products" is being prepared for a book entitled HALOGENATED BIPHENYLS, TERPHENYLS, NAPHTHALENES, DIBENZODIOXINS, AND RELATED PRODUCTS. This work is continuing as different chemicals become available.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There has been considerable human exposure to PBBs and PCBs (polychlorinated biphenyls). These experiments were designed to analyze the structure-activity relationships of PCBs and PBBs. Toxicity appears to correlate well with structure-activity relationships. In particular, those compounds which induce P-448 are the most toxic.

PUBLICATIONS

Goldstein, J.A. The structure-activity relationships of halogenated biphenyls as enzyme inducers. NY Acad. Sci. (in press).

Goldstein, J.A., Hass, J.R., Linko, P. and Harvan, D.J. 2,3,7,8-tetrachlorodibenzofuran in a commercially available 99% pure polychlorinated biphenyl isomer identified as the inducer of hepatic cytochrome P-448 and aryl hydrocarbon hydroxylase in the rat. *Drug Metab. Dispos.* *6*: 258-264, 1978.

Goldstein, J.A., Linko, P.C., Levy, L.A., McKinney, J.D., Gupta, B.N. and Moore, J.R. A comparison of a commercial polybrominated biphenyl mixture 2,4,5,2',4',-5'-hexabromobiphenyl and 2,3,6,7-tetrabromonaphthalene as inducers of liver microsomal drug metabolizing enzymes. *Biochem. Pharmacol.* (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30085-02 EBB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Early Detection of Lung Injury

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. G. Tombropoulos	Research Chemist	EBB NIEHS
	E. W. Van Stee	Physiologist	EBB NIEHS
OTHER:	W. Gibson	Chemist	EBB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology Branch

SECTION
Inhalation Toxicology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pulmonary lavage is easily obtainable and can be taken at various time intervals from the same animal (large such as dogs) without killing the animal. Pulmonary lavage contains lipoproteins, lipids, proteins, cells including macrophages and enzymes. The majority of these components are synthesized by the lung but some come via the pulmonary circulation, and some originate from bone marrow such as the macrophages. The alveolar macrophages are vital entities of the mammalian immune mechanism (in the latter, capacity macrophages depend upon plasma membrane activity for migration recognition, activation, phagocytosis and attachment to substrate tissue).

Thus lavage can be used as a probe for early detection of lung damage, and of lung immune mechanism.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Rabbits were exposed to 250 ppm morpholine vapor for 6 hrs per day. The animals were exposed 33 times in whole body exposure chambers. Control animals were placed in similar chambers for the same duration as the morpholine exposed.

At the end of the experiment both groups, the morpholine and sham exposed animals were divided into two subgroups; the one subgroup was killed immediately after the last exposure and the other subgroup two days later. Lung lavages were obtained, centrifuged and separated into supernatant and cellular material (macrophages). Enzymatic activities were measured in both the supernatant and the macrophage fractions after their disruption by sonication. The enzymes measured were: acid phosphatase, alkaline phosphatase, β-N-acetylglucosaminidase, and α-mannosidase. An aliquot of non-disrupted macrophages was assayed for the binding of wheat germ agglutinin. A part of lung tissue was used for morpholine concentration analysis. Another part of lung tissue was homogenized, fractionated, and the microsomal fraction was used for the assay of P-450 levels.

MAJOR FINDINGS AND PROPOSED COURSE: At the end of the exposure the morpholine concentration in the lung was: male rabbits-93μg/g of tissue; female rabbits-73μg/g tissue and two days after the last exposure the concentration of morpholine was: 77μg/g of lung tissue in the males and 71μg/g lung tissue in the females. Biochemical measurements were made in both groups, those killed immediately after exposure and those killed 2 days postexposure. The measurements made so far have shown great variability which is not unexpected when dealing with marginal expressions of toxicity.

The variability was greater among enzymes measured in the supernatant than those measured in the macrophages. Few significant changes were detected among the variables measured attributable to the chemical treatment. However, we believe that certain trends are evident and their consistency suggests to us that if we were to increase our number of experiments and thus increase the degrees of freedom available to us, more statistically significant responses might be detected. Based on the observations to date we believe that α-mannosidase activity in the alveolar macrophage is increased acutely by exposure to morpholine, but that this effect does not persist to 48 hours postexposure. Acid phosphatase activity was also increased in the macrophages and both enzymes showed a difference between sexes. Further experiments may support the idea that N-acetylglucosaminidase activity is significantly increased by morpholine. The tendency seems to be there but it is significant only at the 10% level. Males had a tendency to respond to a greater extent than females and the opposite was true for the binding of wheat germ agglutinin. The increased activity of N-acetylglucosaminidase may result in a decrease in the number of N-acetyl function groups in the surface of macrophages with a resulting decrease in the wheat germ agglutinin binding.

The effect of the lectin binding seemed to be related to the extracellular enzyme concentration (supernatant) and not to the intracellular enzymes of the macrophages because the binding of lectin by cultured alveolar macrophages was increased although the N-acetylglucosaminidase tended to increase too.

Macrophages cultured for 20 hours also tend to respond differently depending on the sex of the animals from which they were obtained. Alveolar macrophages obtained from male animals were more reactive to morpholine than alveolar macrophages obtained from female animals. Alpha mannosidase levels in cultured macrophages was the most sensitive indicator of morpholine toxicity among the enzymes.

Thus the effect of morpholine on lung macrophages in vivo and in vitro agree. In both cases α-mannosidase activity was the most pronounced index of toxicity and the degree of response depended on the sex of the animal from which the macrophages were obtained.

Another variable studied was P-450 activity in the lung. P-450 lung microsomes decreased and the females showed a greater response than males. The decrease of P-450 levels persisted for at least 2 days postexposure. The decrease was not only per milligram of protein, but also per total lung. The decreased P-450 activity may be indicative of impairment of the MFO system of the lungs.

In conclusion, lavages obtained from male animals showed more pronounced changes in enzymic levels as a result of morpholine inhalation than lavages obtained from female animals. The most significant enzymic alteration was that of α-mannosidase which increased from 50% to 200%. Preliminary studies suggest that alterations in lectin binding by alveolar macrophages may be associated with alterations of extracellular enzyme activities rather than intracellular enzyme activities of the macrophages. No histopathological changes were observed at the low levels of exposure associated with the enzymic changes. Decreases in P-450 activity observed in the lung tissue persisted longer than changes in enzyme levels observed in the lavage. Any potential relationship between these observations and lung chemical biotransformation activity remains unknown at this time.

The studies reported here have required a substantial effort, not only because of the analytical work done, but also because of the difficulty in exposing large numbers of rabbits by inhalation. We know from the variability encountered in the measurements made that we must increase our sample size substantially in order to have a reasonable chance of detecting significant effects in our model systems. As an alternative to this, or perhaps better, as a complement to the inhalation studies, we are studying the possibility of using in vitro tests based on tissue or cell culture models to provide guidance in where to go with the more cumbersome inhalation experiments.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A great need exists for the development of indices of early toxic injury to the lung. The lung is a central organ in matters related to inhalation toxicology. The lung serves the function of portal of entry, site of biotransformation, and an organ of elimination for many compounds. Soluble compounds to which the organism is exposed by inhalation may or may not have a deleterious effect on the lung itself. The lung may constitute the organ through which the parent compound and/or its products of biotransformation are introduced into the organism with the result of the production of tissue injury to the lung itself or other organs of the body. The objective of this project is the search or evidence of early toxic injury that may shed light on subsequent events.

PUBLICATIONS

Hubbard, W.D., Prosser, A.R., Sheppard, A.J., Newkirk, D.R., Osgood, T., Tombropoulos, E.G. and Johns, S.T. Individual lipids and proximate analysis of various foods. I. French fried potatoes from ten chain restaurants. Journal of Agric. and Food Chem. Vol. 25: 280-281, 1977.

Hook, G.E.R., Gilmore, L.B., Tombropoulos, E.G. and Fabro, S.E. Fetal lung lamellar bodies in human amniotic fluid. Am. Rv. of Resp. Dis. Vol. 117: 541-550, 1978.

Tombropoulos, E.G. Micromethod for the gas chromatographic determination of morpholine in biological tissues and fluids. J. Chromatog. (in press).

Tombropoulos, E.G. and Van Stee, E.W. Tissue distribution of inhaled morpholine and its effects on lung lavages. Fed. Proc. 38: 583, 1979.

Kung, H.C., Tombropoulos, E.G. and Wilson, A.G.E. Effect of pre-exposure of rabbits to morpholine on imipramine pharmacokinetics in the isolated perfused lung (IPL). Proc. SOT 18th Ann. Mtg., p A65, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30088-01 EBB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Toxicity Bioassay of Chlorodecone (Kepone)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.A. Moore	Supervisory Veterinary Medical Officer	EBB NIEHS
OTHER:	E.E. McConnell	Veterinary Pathologist	EBB NIEHS
	M.I. Luster	Research Chemist	ECB NIEHS
	G.A. Boorman	Veterinary Pathologist	EBB NIEHS
	M.W. Harris	Biological Laboratory Technician	EBB NIEHS

COOPERATING UNITS (if any)

H. Tilson, LBNT, NIEHS (see LBNT report for details)
J. Hall, LET, NIEHS

LAB/BRANCH

Environmental Biology Branch

SECTION

Comparative Pathology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.8	PROFESSIONAL: 0.5	OTHER: 1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The object of this study was to determine the toxicity of chlorodecone (kepone) in male and female Fischer 344 rats. Kepone was administered via feed for 51 days at doses of 0, 3, 10, 30 and 100 ppm. Parameters of toxicity studied included effects on fertility and reproduction, neurobehavior, immune response, food consumption, body weight gain, hematology and clinical chemistry, and macro- and histopathology.

PROJECT DESCRIPTION

OBJECTIVE: The purpose of this study was to study various toxicologic effects of chlorodecone (kepone) in rats.

METHODS EMPLOYED: Male and female Fischer 344 rats were exposed to kepone via feed for 51 days at doses of 0, 3, 10, 30, and 100 ppm. During exposure parameters studied were body weight gain, feed consumption and clinical appearance. At the end of exposure randomly selected animals were killed for macro- and histopathology, organ weights, hematology and clinical chemistry. Selected tissues were preserved for chemical analysis. Other animals were selected for fertility and reproduction studies and to assess neurobehavioral effects.

MAJOR FINDINGS AND PROPOSED COURSE: The data is incomplete as of May 1, 1979. The study is to be continued.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Kepone was responsible for a large environmental catastrophy in Virginia. Factory workers and their families were exposed to high levels of the chemical with resultant health problems. A large area of the James River and its aquatic life is still highly contaminated with the potential of further human exposure. More definitive information is needed in regard to this chemical, especially in regard to dose response, fertility effects and neurobehavioral effects.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30089-01 EBB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Efforts to Enhance the Elimination of Polybrominated Biphenyls (Firemaster FF-1) from the Body of Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E.E. McConnell	Veterinary Pathologist	EBB NIEHS
OTHER:	J.A. Moore	Supervisory Veterinary Medical Officer	EBB NIEHS
	M.W. Harris	Biological Laboratory Technician	EBB NIEHS
	R.E. Wilson	Biological Laboratory Technician	EBB NIEHS

COOPERATING UNITS (if any)

J.D. McKinney, ECB, NIEHS

LAB/BRANCH
Environmental Biology Branch

SECTION
Comparative Pathology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.2	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The object of this study was to determine if activated charcoal or cholestyrimine had any effect on enhancing the rate of elimination of polybrominated biphenyls (Firemaster FF-1) from the body of rats.

PROJECT DESCRIPTION

METHODS EMPLOYED: Fischer strain female rats were given 1 mg/kg polybrominated biphenyls (Firemaster FF-1) in their diet daily for 6 months. They were then continued on a control diet (no exposure) for 3 months. Following this the animals were divided into groups of 6 (exposed) or 9 (controls) and were given charcoal or cholestyrimine in their diet with or without periods of restricted caloric intake as follows:

Group I	Control	rats	Normal	diet	
II	"	"	"	"	and charcoal
III	"	"	"	"	and cholestyrimine
IV	"	"	"	"	and periods of restricted food intake
V	FF-1	"	"	"	
VI	"	"	"	"	and charcoal
VII	"	"	"	"	" " and restricted calories
VIII	"	"	"	"	and cholestyrimine
IX	"	"	"	"	" " and restricted calories

After six months on the above dietary regimen, the animals were killed. Parameters evaluated were body weight gain, organ weights, hematology and clinical chemistry, and chemical analysis of fat and liver for PBBs.

MAJOR FINDINGS AND PROPOSED COURSE: Data is incomplete as of May 1, 1979. This project is to be continued.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: PBBs were responsible for widespread environmental contamination, animal loss and suspected human illness in Michigan during 1973-1974. People in this area still have body burdens of PBBs. It would be extremely useful to find a therapeutic compound which would enhance the elimination of these chemicals from the body.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30090-01 EBB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Toxicologic Evaluation of Pentachloranisole

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.L. Wagner	Visiting Scientist	EBB NIEHS
OTHER:	J.A. Moore	Supervisory Veterinary Medical Officer	EBB NIEHS
	E.E. McConnell	Veterinary Pathologist	EBB NIEHS
	M.W. Harris	Biological Laboratory Technician	EBB NIEHS

COOPERATING UNITS (if any)

Division of Laboratory Animal Resources, Duke University, Durham, NC

LAB/BRANCH
Environmental Biology Branch

SECTION
Comparative Pathology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The object of this study is to evaluate the acute and subchronic effects of pentachloranisole in Fischer-344 rats and B6/C3F1 mice.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Male and female B6/C3F1 mice and Fischer-344 rats will be exposed to various doses of pentachloranisole via gavage to determine the acute LD_{50}. Subsequently, based on this study, mice and rats will be exposed to the compound for 90 days. The toxicologic parameters being evaluated are clinical appearance, body weight gain, hematology and clinical chemistry, organ weight effects, and macro and histopathology. This study will be completed in FY 79.

MAJOR FINDINGS AND PROPOSED COURSE: The study is scheduled to start May 1, 1979.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Pentachlorophenol (PCP) is the second most commonly used insecticide in the United States. In a national survey, 85% of urine samples examined from the human population contained traces of PCP. The toxicologic profile of this compound (PCP) has been established; however, a metabolite of this compound, namely PCA has not yet been subjected to toxicologic evaluation. It is the intent of this study to determine the acute and subacute toxicity of PCA in rats and mice.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30091-01 EBB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

A Comparison of Low Level Exposure of Polybrominated Biphenyls (Firemaster FF-1) in the Diet vs Gavage in Male CDF Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.W. Harris	Biological Laboratory Technician	EBB NIEHS
	E.E. McConnell	Veterinary Pathologist	EBB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Environmental Biology Branch

SECTION
Comparative Biology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2 technical

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study in male CDF rats was to compre the effects of a low level of polybrominated biphenyls (Firemaster FF-1) administered in the diet with the same level via gavage. In addition, to compare the rate of body weight gain in nonexposed rats given powdered diet versus the same diet in a pelleted form.

PROJECT DESCRIPTION

METHODS EMPLOYED: Male CDF rats, 6-7 weeks of age were fed ground NIH rodent chow containing Firemaster FF-1 at 1 mg/kg/day. Food consumption was measured 3x weekly; body weight changes were measured once weekly. The control rats being fed either ground or pelleted chow were handled in the same manner. All rats were fed ad libitum for 6 months.

MAJOR FINDINGS AND PROPOSED COURSE: There was no significant difference in the effects of FF-1 between the two routes of exposure in the parameters measured. Additionally there was no difference between the control animals fed ground diet compared to pelleted diet.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Administration of test substances to a large group of animals via the diet requires less technical time than does daily gavage. In long-term chronic exposure studies this is of importance. The relative ease of adding test substances to ground feed as opposed to pelleting after the addition of test substances is also of great time-saving value in toxicological testing.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30092-01 EBB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Effect of Biphenyls on Bone Marrow Cellularity and Progenitor Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G.A. Boorman	Veterinary Pathologist	EBB NIEHS
OTHER:	M.I. Luster	Research Chemist	ECB NIEHS
	J.A. Moore	Supervisory Veterinary Medical Officer	EBB NIEHS
	M.W. Harris	Biological Laboratory Technician	EBB NIEHS
	J.A. Allen	Biological Laboratory Technician	EBB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Environmental Biology Branch

SECTION
Comparative Pathology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The object of this study is to determine if polybrominated biphenyls (PBB) or tetrachlorodibenzodioxin (TCDD) have any effect on bone marrow cellularity, macrophage-granulocyte progenitors (CFU-C) or pleuripotent bone marrow stem cells (CFU-S).

PROJECT DESCRIPTION

METHODS EMPLOYED: B6C3F1 mice were given polybrominated biphenyl (Firemaster FF-1) at 0, 1, 10 mg/kg/day for 6 months or maternal dosing at 0, 1, 3, 10 mg/kg every other day (total 22 doses) until weaning. At necropsy the femors were removed, marrow flushed from the shaft, cells counted and plated in semisolid methylcellulose cultures and injected into irradiated recipients for spleen colony assay (CFU-S). Tetrachlorodibenzodioxin (TCDD) was given maternally on gestation day 14, and days 1, 7, and 14 following parturation at 0, 1, 5 and 15 mg/kg body weight.

MAJOR FINDINGS AND PROPOSED COURSE: In maternal and adult PBB exposure no effect or pleuripotent stem cells (CFU-S) or bone marrow cellularity was found. Enhancement of colonies in methylcellulose cultures (CFU-C) was found in adult females exposed to 1 to 10 mg/kg/day. In TCDD exposed mice the 15 μg/kg group had 69% reduction in pleuripotent stem cells as well as reduction in bone marrow cellularity. The 5 μg/kg group showed a 29% reduction in bone marrow stem cells.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Earlier studies from this Institute have shown decreased bone marrow cellularity (noted on histological preparations) and altered hematological parameters. This study provides a more precise quantitation of bone marrow cellularity and identifies specific cell populations involved. These studies help evaluate new methods of assessing environmental toxicity as well as providing a clearer understanding of their mechanism of action.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30093-01 EBB

PERIOD COVERED
October 1, 1978 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Macrophage Function and Environmental Toxicology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G.A. Boorman	Veterinary Pathologist	EBB NIEHS
OTHER:	M.I. Luster	Research Chemist	ECB NIEHS
	R.E. Wilson	Biological Laboratory Technician	EBB NIEHS

COOPERATING UNITS (if any)

J. Thigpen, CMB, NIEHS

LAB/BRANCH
Environmental Biology Branch

SECTION
Comparative Pathology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The object of this study is to develop a panel of tests that will assess macrophage function in animals exposed to toxic environmental agents.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Pulmonary alveolar macrophages, resident peritoneal macrophages, and thioglycollate induced peritoneal exudate cells are collected from control B6C3F1 female mice and those exposed to polybrominated biphenyl (PBB) and tetrachlorodibenzodioxin (TCDD) and tested for a variety of parameters that involve macrophage function. Specifically macrophages are assessed for acid phosphatase content, ability to phagocytize sheep red blood cells and numbers of macrophages. In addition, two new assays are being studied. A microtiter method for measuring macrophage proliferation by tritiated thymidine uptake has proved to be easy and reproducible. Macrophage cytotoxicity as measured by release of tritiated thymidine from labeled tumor cells is under development. General macrophage tests such as host resistence to Listeria monocytogenes and endotoxin sensitivity are also used.

MAJOR FINDINGS AND PROPOSED COURSE: Mice exposed to PBB show marginal increased sensitivity to Listeria and increased endotoxin sensitivity. Acid phosphatase content and phagocytosis by macrophages are not altered. The effect of TCDD and PBB on other macrophage parameters has not been completed as of May 1, 1979. Study is to be continued.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PURPOSE OF THE INSTITUTE: This Institute is committing a major effort to understanding the effect of environmental agents on the immune system. The macrophage plays a central role in the immune system by interacting with lymphocytes and secreting monokines. Thus an understanding of immunotoxicology requires a study of the effect of toxic agents on macrophages. Current efforts are directed toward delineating which assays will accurately reflect an alteration in macrophage function.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30094-01 EBB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of Polychlorinated Biphenyl Isomers on Extrahepatic Metabolism of Benzpyrene

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.A. Goldstein	Pharmacologist	EBB NIEHS
	J. Nagayama	Visiting Fellow	EBB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Environmental Biology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.7	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study was initiated to examine the effects of two polychlorinated biphenyl (PCB) isomers (2,4,5,2',4',5'-PCB and 3,4,3',4',5'-PCB and a commercial PCB mixture on the in vitro metabolism of benzpyrene [metabolic profile] of benzpyrene metabolites) and covalent binding of benzpyrene to macromolecules in skin and other extrahepatic tissues.

PROJECT DESCRIPTION

METHODS EMPLOYED: CD-1 mice were treated cutaneously with Aroclor 1254 and the metabolism of benzpyrene examined by high pressure liquid chromatography (HDLC) and standard techniques for determining fluorescent products.

MAJOR FINDINGS AND PROPOSED COURSE: Conditions for assay in skin were checked and the linear range established. Aroclor 1254 induces the formation of fluorescent products. HPLC conditions were set up and preliminary examinations of benzpyrene metabolites in skin underway. Assay of total metabolites formed, profile of metabolites, and epoxide hydrase activity in this and other extrahepatic tissues may be followed. Only preliminary data has been obtained by HPLC. This project may be continued, dependent on personnel.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: PCB mixtures affect the metabolism of carcinogens such as benzpyrene in hepatic and extrahepatic tissues. PCBs concentrate in skin, even when given orally, but induction by PCBs in this and other extrahepatic tissues has received little attention. Since both benzpyrene and PCBs are widespread contaminants, PCBs could influence the metabolism of BP to the ultimate carcinogenic metabolites. Tumor initiation or promotion could thus be influenced.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30095-01 EBB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of Cutaneous Administration of Technical Pentachlorophenol on Hepatic Enzymes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Linko	Chemist	EBB NIEHS
	J.A. Goldstein	Pharmacologist	EBB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Environmental Biology Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.6	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study was initiated to determine whether exposure to technical pentachlorophenol (tPCP) by the cutaneous route affects the liver; to compare the effectiveness of the cutaneous versus the oral route on hepatic mixed function oxidases (MFO's); and to compare pure versus technical pentachlorophenol to ascertain whether effects on MFO's are due to pentachlorophenol or to its contaminants, the dibenzodioxins and dibenzofurans.

PROJECT DESCRIPTION

MAJOR FINDINGS AND PROPOSED COURSE: Technical PCP, like its dibenzodioxin contaminants increased hepatic aryl hydrocarbon hydroxylase (AHH) and the amount of cytochrome P-448 in the liver. Pure PCP does not affect MFO's. Cutaneous exposure to technical PCP induces hepatic enzymes in both the rat and mouse but the oral route is more effective. Preliminary work in the rat and mouse indicated that the commonly used methods of dermal exposure (application to the nuchal area or between the shoulder blades) results in a considerable amount of oral ingestion. Data from unpatched animals resulted in much greater "apparent dermal" effects than data from patched animals even when the material was carefully placed, indicating that studies of the systemic effects or absorption of compounds via the dermal route utilizing unpatched mice or rats are invalid.

In subsequent work, we covered the exposed area with a patch which was attached to the skin with cyanoacrylic glue. Dose response data indicated that cutaneous exposure to technical PCP does have systemic effects, but compared to oral dosing, a 10-fold higher cutaneous dose is required to produce an equivalent effect. This study is complete except for the final data tabulating and report which will be completed in FY 1980.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Pentachlorophenol is widely used as a wood preservative in the lumber and building industries. Many products are contaminated with dibenzodioxins since there are no current regulations concerning the presence of these highly toxic compounds. Human exposure is primarily cutaneous. This study is the first to demonstrate that this compound does have systemic effects even after dermal exposure.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30096-01 EBB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Use of Whole-Body Sagittal Histologic Sections in Teratologic Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B.N. Gupta	Veterinary Pathologist	EBB NIEHS
	J.A. Moore	Supervisory Veterinary Medical Officer	EBB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Environmental Biology Branch

SECTION
Comparative Pathology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Histologic sections of whole body mouse fetus were evaluated as an aid in teratologic studies. A technique was developed in which 3 subserial sagittal sections from each fetal mouse were examined histologically. Using this method lung, liver, pancreas, intestines, salivary gland, brain and kidney were present in the sections over 97% of times. Other organs were present in the following order: heart 88%; thymus 74%; adrenal gland 72%; pituitary and ethyroid glands 63%; and parathyroid glands only 14%. This technique may be used to evaluate the microscopic alterations in different organs of the mouse fetus in teratologic or fetal toxicity studies. The purpose of this study was to develop a technique to aid in teratologic or fetal toxicity studies by microscopic observations of different organs.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Mouse fetuses obtained by cesarian section on day 18 of gestation were placed in bottles containing buffered neutral 10% formalin. After 3 to 4 hours, 0.2 ml of the formalin solution was injected intraperitoneally for better fixation of the visceral organs. Several millimeters thick, skin and subcutaneous tissues of each fetus were sliced off sagitally from both left and right sides. The trimmed fetuses were routinely embedded in paraffin for microscopic examination. Three subserial sagittal sections, one each from the left, middle and right side of the body were cut at 6 μm and stained with hematoxylin and eosin. For preliminary evaluation 136 fetuses were examined.

MAJOR FINDINGS AND PROPOSED COURSE: Most vital organs such as lung, liver, kidney, heart and brain were present in the whole body sections. Summary of the detailed organ findings and their 95% confidence limits are given in Table 1. Multiple midline sections of the fetal head might be more appropriate than the whole body sections to study the pituitary gland. Similarly, if one wants to examine the thyroid or parathyroid glands, transverse sections of neck near the tracheo-laryngeal area will prove more useful. Thus the percentage rate of true success of a given organ, such as parathyroid (14%) and spleen (37%) may be greatly increased with a slight modification of this technic. This study is to be continued.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Traditionally, teratological studies involve the examination of different organs or body systems of fetus for malformations. The examination is usually conducted visually or under a dissecting microscope. This technic of microscopic examination of the fetus in one to three sections may aid in teratologic or fetal toxicity studies.

TABLE 1

Percentages of Different Organs Observed in
136 Mouse Fetuses on Slide Sections

Organs	Observed (P) success rate		Percentage	95% Confidence limits on true success rate P
Lung, liver, pancreas, small intestine	136	100		0.978 - 1.000
Salivary gland	135	99.3		0.960 - .9999
Cerebrum	135	99.3		0.960 - .9999
Large intestine	134	98.5		0.947 - .998
Nasal turbinate	132	97.1		0.926 - .992
Cerebellum	132	97.1		0.926 - .992
Kidney	129	94.9		0.897 - .979
Heart	119	87.5		0.807 - .926
Spinal cord	114	83.8		0.765 - .896
Tongue	113	83.1		0.757 - .890
Olfactory lobe	112	82.4		0.749 - .884
Ear	110	80.9		0.732 - .871
Thymus	101	74.3		0.661 - .814
Adrenal	98	72.1		0.637 - .794
Urinary bladder	98	72.1		0.637 - .794
Stomach	93	68.4		0.599 - .761
Gonad	92	67.6		0.591 - .754
Pituitary	86	63.2		0.545 - .713
Thyroid	85	62.5		0.538 - .707
Eye	59	43.4		0.349 - .522
Spleen	50	36.8		0.287 - .455
Parathyroid	19	14.0		0.086 - .210

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30097-01 EBB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Fetal and Maternal Toxicity of 3,3',4,4',5,5'-Hexachlorobiphenyl in the Mouse .

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B.N. Gupta	Veterinary Pathologist	EBB NIEHS
	R.E. Staples	Research Physiologist	EBB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Environmental Biology Branch

SECTION
Comparative Pathology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A symmetrical isomer of polychlorinated biphenyl, (PCB), 3,3',4,4',5,5'-hexachlorobiphenyl (HCB) was given to pregnant mice on the 6th through 15th days of pregnancy @ 0, 0.1, 1.0, 2.0, 4.0, 8.0 or 16.0 mg/kg body weight. At sacrifice on day 18 of gestation, pathologic changes in the maternal and fetal liver were greatest but the microscopic pattern was remarkably different. Changes in the maternal liver were characterized by moderate to marked swelling of hepatocytes, single cell necrosis primarily around the central veins and occasional microabscesses. On the contrary, in the fetal liver there was massive necrosis in the hepatic lobes and formation of a tubule-like pattern of hepatocytes. The tubule-like formation was similar to the hepatotoxic lesions induced by other halogenated polycyclic hydrocarbons in the adult rats. The objective of this study was to find out the toxicity of a pure isomer of PCB (3,3',4,4',5,5'-hexachlorobiphenyl) in the fetus and the pregnant mouse.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Pregnant mice were given 3,3',4,4',5,5'-hexachlorobiphenyl (HCB) by gavage at daily dosages of 0, 0.1, 1.0, 2.0, 4.0, 8.0 or 16.0 mg/kg body weight from days 6 through 15 of gestation. The control mice were given cotton seed oil only. All mice were sacrificed by cervical dislocation on day 18 of gestation. The fetuses were removed by cesarian section and whole body was fixed in buffered neutral 10% formalin. After few hours, about 0.2 ml of the formalin fixative was also injected intraperitoneally into each fetus for better fixation of the visceral organs. Several millimeters of skin and subcutaneous tissues were sliced off sagitally from both the left and right sides and then routinely embedded in paraffin for histopathologic examination. Three subserial sections, one on each left, middle and right side of the body were prepared sagitally. Liver, heart, kidney, spleen and thymus from the dam were also collected randomly for histopathologic evaluation.

MAJOR FINDINGS AND PROPOSED COURSE: The fetal livers from treated mice appeared slightly enlarged and pale with an accentuated lobular artitecture. Occasionally, a part or whole lobe of the liver appeared pale or gray with distinct border. The massive necrosis of the liver was observed primarily in mice from dams given 4.0 mg/kg or more of HCB.

Microscopic changes in the livers of pregnant mice given a daily dose of HCB @ 8.0 and 16.0 mg/kg were characterized by marked swelling of hepatocytes, hydropic degeneration, fatty infiltration, pyknotic nuclei and karyolysis. There was necrosis of hepatocytes primarily around the central veins with increased neutropholic infiltration into the perivascular spaces. Eosinophilic hyaline bodies were observed in some degenerating hepatocytes. Increased mitosis of hepatocytes was observed and the nuclei of these dividing cells were affected primarily. This study will be completed in FY 79.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Polychlorinated biphenyls (PCB) are found in a wide variety of products such as paints, printing inks, electrical equipment, adhesives and others. PCBs are so widespread that they are believed to be present in the adipose tissues of nearly every person in the United States, Japan and other industrialized countries. Findings of this experiment indicated that the fetal toxicity resulted from the placental transfer of PCB into the developing fetus. This suggests that pregnant women with high levels of PCBs in their adipose tissue should be aware that a fat reduction program during gestation may expose the unborn child to PCBs *via* placental transfer.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30098-01 EBB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Tumorigenic Potential of NO_2 Inhalation in Small Animals Exposed to Heterocyclic Amines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E.W. Van Stee	Physiologist	EBB NIEHS
	G.A. Boorman	Comparative Pathologist	EBB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Environmental Biology Branch

SECTION
Inhalation Toxicology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Random bred, male Fisher 344 rats, CD-1 mice, and golden Syrian hamsters were exposed to 1-2 ppm of NO_2 for 6 hr/day, 5 da/wk for 30 weeks. Some of the animals also received 0.1% morpholine in deionized water as their sole source of drinking water. The animals will be held for 50 wk after the 30 wk NO_2 exposure period during which time all animals will receive deionized water. Animals will be killed for histopathological evaluation following the postexposure holding period. Future studies will include other heterocyclic amines, e.g., piperazine, 2,6-dimethylmorpholine.

PROJECT DESCRIPTION

METHODS EMPLOYED: Rats, mice, or hamsters are exposed to NO_2 (N_2O_4) in dynamic, flow-through chambers. Exposures are conducted for 6-7 hr/da, 5-7 da/wk, for 30-104 weeks. Subgroups of animals are given heterocyclic amines in the drinking water. Animals are killed approximately 2 yr from the beginning of the exposures for gross and histopathological examination.

MAJOR FINDINGS AND PROPOSED COURSE: Experiment 1: Male Fisher 344 rats, CD-1 mice, and golden Syrian hamsters were exposed to air or 1-2 ppm NO_2, 6 hr/da, 5 da/wk, for 30 weeks. Part of the animals received 0.1% morpholine in their drinking water. Animals were randomly assigned to each of 4 treatment groups in a 2 x 2 layout: air + deionized water, NO_2 + deionized water, air + morpholine water, and NO_2 + morpholine water. Animals will be killed at the end of 2 yr for pathological evaluation. Only sporadic deaths have occurred so far (4 mo postexposure). Further information will not become available until early 1980. Experiment 2: rats, mice, or hamsters will be exposed to air or 10 ppm of NO_2 6-7 hr/da, 5-7 da/wk, for 104 weeks. They will be assigned to each of 4 groups in a 2 x 2 layout as in Experiment 1. Animals will be killed for pathological evaluation at the end of the 104 wk exposure. Further studies may include the examination of urine from animals, exposure concurrently to NO_2 and heterocyclic amines, for the presence of mutagens. Similar studies may be pursued in vitro in which the heterocyclic amines are added to hepatic or pulmonary microsomes, exposed to NO_2 and the mixture assayed for the presence of mutagens or, more specifically, nitrosamines.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Nitrosamines have been demonstrated to be formed in vitro at neutral pH by mixing heterocyclic amines like morpholine and NO_2 in aqueous media, e.g. plasma. These reactions have the potential for yielding nitrosamines known to be potent animals carcinogens, in vivo. The concept is under consideration for extensive evaluation through the National Toxicology Program. The general hypothesis to be tested is that animals concurrently exposed by inhalation to NO_2 and heterocyclic amines by any route will carry increased total body burdens of nitrosamines whose presence, at some concentrations, will be expressed as nitrosamine toxicity, principally oncogenesis.

ILLINOIS INSTITUTE OF TECHNOLOGY RESEARCH INSTITUTE
Chicago, Illinois 60616
NIEHS-NO1-ES-5-2157

TITLE: Long Term Study on the Effect of Ingested Asbestos in Hamsters

CONTRACTOR'S PROJECT DIRECTOR: Alan M. Shefner, Ph.D.

PROJECT OFFICER (NIEHS): J. A. Moore, D.V.M., Deputy Director, National Toxicology Program

DATE CONTRACT INITIATED: June 30, 1975

CURRENT ANNUAL LEVEL: $300,000

PROJECT DESCRIPTION

OBJECTIVES: This contract is for the purpose of studying the long term effects of ingestion (via feed) of asbestos in hamsters. Types of asbestos fibers being studied are short range (fiber size chrysotile, intermediate range chrysotile and amosite. In addition, low levels of 1,2-Dimethylhydrazine (a known intestinal carcinogen) are being used in conjunction with intermediate range chrysotile to study its co-carcinogenic potential.

METHODS EMPLOYED: The above asbestos fibers are mixed in the food at the rate of 1 percent in the diet and the male and female hamsters are fed this diet for their lifetime. Parameters being evaluated are body weight gain, clinical effects, and most importantly, the macro- and histopathology observed at death.

MAJOR FINDINGS AND PROPOSED COURSE: As of May 1, 1979, the various groups have been on test for 88 to 109 weeks. 99+ percent of the females (all groups) and 60+ percent of the males have died to date. Results to date suggest that there was no effect of asbestos on body weight gain. Other results are not complete enough to evaluate. The proposed course for the immediate future is to complete all studies on the female animals before analyzing the male hamsters.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The inhalation of asbestos is known to cause cancer in both man and experimental animals. However, the hazards from ingestion of asbestos are unknown. Surveys have shown that the water supplies of several metropolitan areas in the United States are contaminated with asbestos fibers as are several common food items. Because of this it is extremely important to evaluate the effects of ingested asbestos in experimental animals.

HAZLETON LABORATORIES AMERICA, INC. - Vienna, Virginia 21180
NIEHS-N01-ES-5-2158

TITLE: Long Term Study on the Biological Effects of Ingested Asbestos in Rats

CONTRACTOR'S PROJECT DIRECTOR: Henry A. Rutter, Ph.D.

PROJECT OFFICER (NIEHS): J. A. Moore, D.V.M., Deputy Director, National Toxicology Program

DATE CONTRACT INITIATED: June 30, 1975

CURRENT ANNUAL LEVEL: $450,000

PROJECT DESCRIPTION

OBJECTIVES: This contract is for the purpose of studying the long term effects of ingestion (via feed) of various types of asbestos fibers in rats. The types of asbestos fibers being studied are medium range chrysotile, short range chrysotile, tremolite, crocidolite and amosite. In addition, low levels of 1,2-Dimethylhydrazine (a known intestinal carcinogen) are being used in conjunction with amosite to study its co-carcinogenic potential.

METHODS EMPLOYED: The above fibers are mixed in the food at a rate of 1 percent in the diet and the male and female rats are fed this diet for their lifetime. Parameters being evaluated are body weight gain, clinical effects and most importantly, the macro- and histopathology observed at death.

MAJOR FINDINGS AND PROPOSED COURSE: As of May 1, 1979, most of the animal groups have been on test less than 6 months. Therefore, little data is available at this time. This lifetime study will require a further two years before completion.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The inhalation of asbestos is known to cause cancer in both man and experimental animals. However, the hazards from ingestion of asbestos are unknown. Surveys have shown that the water supplies of several metropolitan areas of the United States are contaminated with asbestos fibers as are several common food items. Because of this, it is extremely important to evaluate the effects of ingested asbestos in experimental animals.

BECTON-DICKINSON AND COMPANY - Research Triangle Park, North Carolina 27709
NIEHS N01-4-2164

TITLE: Effects of Chronic Exposure to Airborne Environmental Agents

CONTRACTOR'S PROJECT DIRECTOR: C. J. Stone, Ph.D.

PROJECT OFFICER (NIEHS): J. A. Moore, D.V.M., Associate Director,
Research Resources Program

DATE CONTRACT INITIATED: April 29, 1974

CURRENT ANNUAL LEVEL: $600,000

PROJECT DESCRIPTION

OBJECTIVES: To conduct studies on the inhalation toxicology of environmental chemicals. This involves both selected gases and solid particulates. During this reporting period, studies were conducted on vinyl chloride, various forms of asbestos and glass wool used for insulation.

METHODS: The vinyl chloride study was designed to compare the carcinogenic response in female hamsters, rats and two strains of mice to various dosing regimens. All animals were exposed to 200 ppm VC, 6 hours/day, 5 days/week; the only difference being the length (months) of exposure and the animals age during exposure. The different groups were exposed as follows: 0-6 months of age, 0-12 months, 0-18 months, life-time (24 months), 6-12 months, 6-18 months, 12-18 months, 12-24 months, and 18-24 months.

The fiber (asbestos and glass wool) studies were conducted using male and female Fischer 344 rats which were exposed for 7 hr/day, 5 days/week for 12 months. The animals will then be held for their life-time. The types of asbestos used were short range chrysotile, chrysotile B, and NIEHS intermediate; the glass wool was Johns-Manville 100. The object of the study was to determine and compare the fiber retention in the lung and fibrogenic and carcinogenic potential of these fibers using standard physiological and pathology techniques. An additional morphometric study using electron microscopy is being conducted to study and quantitate the early changes in the lung produced by these fibers.

MAJOR FINDINGS AND PROPOSED COURSE: The results from both studies are incomplete as of May 1, 1979. Preliminary results suggest that there is a marked difference in the carcinogenic response of VC depending on the age of the animal during exposure. For instance, exposure starting at weaning for 6 months caused a higher rate of tumor induction than exposure from 6-12 or 12-18 months. In addition, it appears that the 0-6 months exposure period yielded about as high a tumor incidence as exposure for 0-24 months (life-time). This study will be completed in FY 79.

The results of the fiber studies are much more preliminary. The 12-month exposure was completed in February 1979. Physiological tests and necropsy of selected animals at this time revealed significant effects in the animals exposed to all types of asbestos while those exposed to glass wool were much less affected. At this time no evidence (macroscopic) of neoplasia was noted. The rats will be allowed to live out their normal lifespan which will mean that the study should be completed in the fall of 1980.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The objective of the vinyl chloride study was to evaluate various exposure regimens using a known carcinogen. With knowledge gained from this study, it is hoped that in the future inhalation studies can be better designed in an effort to better utilize the limited resources available to inhalation toxicologists. For instance, this information might allow shorter exposures, thereby significantly reducing the expense of such studies.

The information gained from the asbestos studies will have immediate societal impact since the problems associated with the inhalation of asbestos in humans are well known. One of the major forms of insulation presently being used is glass wool; therefore, the information gained from this aspect of the study is extremely important.

BATTELLE COLUMBUS LABORATORIES - Columbus, Ohio 43201
NIEHS-N01-ES-8-2151

TITLE: Comparative Carcinogenicity and Toxicity Studies of Selected Environmental Chemicals in Laboratory Animals Exposed During Pre- and Postnatal Life

CONTRACTOR'S PROJECT DIRECTOR: Arthur C. Peters, D.V.M.

PROJECT OFFICER (NIEHS): Rajendra S. Chhabra, Ph.D., Pharmacologist
Environmental Biology Branch

DATE CONTRACT INITIATED: September 30, 1978

CURRENT ANNUAL LEVEL: $308,954.00

PROJECT DESCRIPTION

OBJECTIVES: The main objective of this study is to determine if there is increased sensitivity of laboratory animals to potential carcinogenic and toxicologic effects of selected environmental chemicals exposed at various dose levels during their in utero development plus postnatal life of two years as compared to the animals exposed only during post-weaning time of two years. This objective will be achieved by (1) studying the carcinogenic potential of selected environmental chemicals exposed to the laboratory animals during their in utero development and weaning period (by treating their mothers with the chemical until weaning) followed by life time exposure (2 years) and comparing it with the effects observed in parallel groups of animals (derived from untreated mothers) exposed to the chemicals only after weaning for two years; (2) studying the effects on reproduction, behavioral, endocrine, weaning and immunological functions at specific time periods during the bioassay in additional groups of animals (incorporated in the bioassay design); and (3) the bioassay will be carried out at 3 dose levels plus controls to determine if there is a dose-response relationship of the chemical for carcinogenic and other toxicologic end points.

METHODS EMPLOYED: Under this contract four chemicals are being studied in both male and female B6C3F1 mice and F344 rats. The project is divided into two phases, i.e. prechronic and chronic.

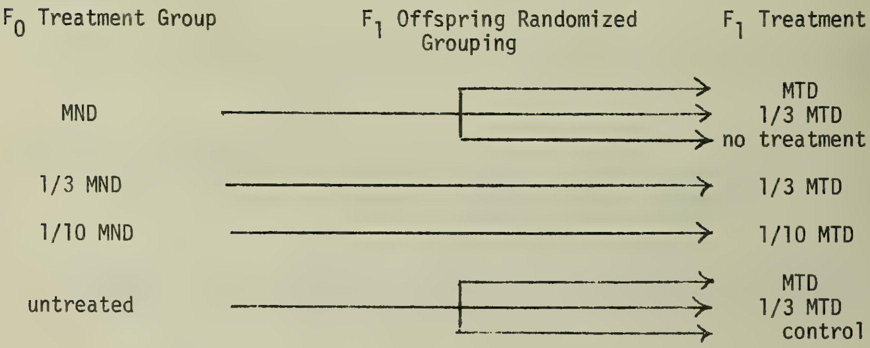
Prechronic Phase of Study: The acute toxicity and MTD (Maximum Tolerated Dose) determination is carried out by following the "Guidelines for Carcinogenic Bioassay in Small Rodents" prepared by the Division of Cancer Cause and Prevention, NCI, NIH.

The Maximum Neonatal Dose (MND) will be determined as follows: mature females (7-9 weeks) will be divided into five groups, i.e. (1) control, (2) MTD (as determined in 90 day studies), (3) 1/3 MTD, (4) 1/10 MTD,

(5) 1/30 MTD. Twelve females per group will be dosed with the chemical (in diet) for four weeks before breeding. These groups will be bred to untreated, proven fertile males after four weeks or to time of steady state for the chemical and continued on the chemical through weaning of the F_1 generation. The size of litters will be limited to 8 in rats and 6 in mice by random killing of excessive animals on day 4. The MND will be the highest dose at weaning which does not depress the body weight of the offspring more than 10% as compared to the controls; and does not produce mortality, clinical signs of toxicity, pathologic lesions or malformations that would be predicted to shorten the animals' natural life span. Extra animals will be added in MND determination experiments to analyze the chemical and/or its metabolite(s) concentrations in the tissues and body fluids by using standard analytical GLC or mass spectrometry methods.

Chronic Phase: In addition to the development of tumors as an end point, the appropriate toxicity tests, general histopathology, behavioral and immunology function tests will also be performed on parallel sets of animals placed on same dose regimen at specific time intervals during the test period. The chronic phase will begin with 4 groups of sexually matured (7-9 weeks) females of both species; groups of 90 animals will receive MND of the chemical; two groups of 30 animals in each will receive 1/3 MND and 1/10 MND respectively; fourth group of 90 animals will not receive any treatment. The dosing will begin 4 weeks later, or to the time of steady state for the chemical, before breeding of all groups. Three days before anticipated delivery, the animals will be transferred to a suitable cage to litter. The F_0 females will continue to receive the test chemical while nursing their litters.

A. Carcinogen Bioassay - At weaning of above four groups, not more than 2 males and 2 females, shall be selected randomly from each litter to obtain the total required for the carcinogen bioassay. Eight groups (16 for both sexes) consisting of 50 offsprings in each (derived from F_0 mothers) will be treated with test chemical for 2 years as outlined below.



MAJOR FINDINGS AND PROPOSED COURSE; Two of the four chemicals were identified for this study just after the award of the contract. These are Phenytoin (Diphenyhydantoin) and Arochlor 1254. Recently ethylenethiourea was selected to be studied under this contract. The selection of the remaining one chemical is still pending. The contractor has finished a survey of literature on Phenytoin and Arochlor 1254. In addition to gaining background information about the chemicals, the survey is expected to: assist in selecting dosages for the preliminary prechronic studies; identify pertinent analytical methods; and facilitate the assessment of literature adequacy in providing metabolism data and estimate of the exposure time required to achieve steady state.

The most time consuming and difficult problem encountered thus far has been related to consideration of the control, containment and disposal of the PCB Arochlor 1254, a necessity to meet the Environmental Protection Agency's regulations for disposal of PCB's. The contractor has prepared a written working plan on various aspects of the impact of changes from the originally proposed study, in terms of methods, effort, and cost estimates and submitted it to NIEHS for its approval.

It is expected that safety procedures for PCB studies will soon be established. However, modifications of the contractor's facilities for this study may take several months before laboratory work on PCB's is initiated. The chemical analysis for identification and purity of Arochlor 1254 and Phenytoin is being performed by Midwest Research Institute and Radian Corporation. The prechronic toxicity studies on Phenytoin are initiated. The procurement of Ethylenethiourea and its chemical purity determinations will soon follow. The selection of the fourth chemical is underway which should help the contractor in planning to complete this project within five years of contract life. It is expected that within next year all prechronic phases will be completed and chronic phases initiated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The discovery of cancer in the daughters of women exposed to diethylstilbesterol, toxicity in babies exposed to hexachloraphene, or morphologic and functional impairments in children whose mothers were exposed to environmental mercury emphasizes the significance of prenatal exposure to chemicals and resulting delayed toxicologic or carcinogenic effects in offspring. The objective of this program is to test the concept that prenatal plus postnatal exposure of chemicals for carcinogen bioassay is perhaps a more sensitive method for assessment of toxicity and carcinogenicity of selected chemicals as compared to widely used approach of lifetime exposure of young adult animals. This research project is directly relevant to the mission of the National Toxicology Program implemented by NIEHS. The data from this research will aid in better risk assessment of the selected chemicals for human exposure for a wide spectrum of the population as well as strengthen the need for different approaches necessary for recognition of reversible or irreversible toxic properties of chemicals in general or for a class of chemicals.

For evaluation of carcinogenic potential the contractor will follow specific toxico-pathologic procedures suggested by NIEHS.

B. General Toxicology Tests - A number of tests will be performed on separate animals incorporated in the carcinogen bioassay design. These animals will be exposed to the test chemical at the same dose regimen as that of carcinogen bioassay groups. Various toxicologic end points to be tested are described below.

I. Toxico-Pathologic Evaluation - A parallel set of 8 groups of each sex shall be set up. These groups will consist of 10 male and 10 female animals at each test level. Each group shall consist of one F_1 male and one F_1 female randomly selected from each 10 litters. These groups will be placed on the appropriate treatment at weaning and sacrificed at 9 months of age for toxico-pathologic evaluations which include gross pathology, histopathology, clinical chemistry and tissue levels of the test chemical.

II. Reproductive Function Tests - The animals of the Toxico-Pathologic group, prior to their sacrifice, will be subjected to reproductive function tests.

III. Immune-Function Tests - One additional set of eight groups will consist of 12 males at each test level. Each group will consist of one F_1 male randomly selected from each of 12 litters at weaning and placed on the appropriate treatment. The animals will be sacrificed at 9 months for immune-function tests such as: T-cell function, *in vitro* by assessing the response of splenic or peripheral blood lymphocytes to mitogen conconavalin A and/or phytohemagglutinin; *in vitro* B-cell function by assessing its *in vitro* response of lymphocytes to poke week mitogen or *E. Coli* lipopolysaccharide; antibody response to T-dependent antigen by plaque assay; delayed hypersensitivity reaction; and quantitation of immunoglobulin.

IV. Behavioral Tests - A battery of behavioral tests will be applied to 10 male rats per treatment groups from 10 separate litters and 20 male controls from separate litters. The behavioral tests will be performed in the same animals at 4 weeks, 9 months, and 2 years of carcinogen bioassay groups. The tests will include spontaneous motor activity; presence or absence of autonomic signs and for the appearance of normal or deferred motor and pain reflexes; visual placement responses; forelimb grip strength; hind limb extensor reflexes; startle responsiveness and habituation to a time-locked acoustic signal; and one-way avoidance response.

OAK RIDGE NATIONAL LABORATORY, U.S. ENERGY RESEARCH AND DEVELOPMENT
ADMINISTRATION - Oak Ridge, Tennessee 37830
(ERDA 40-524-75)

TITLE: Environmental Teratology Information Center (ETIC)

CONTRACTOR'S PROJECT DIRECTOR: John S. Wassom, B.Sc.

PROJECT OFFICER (NIEHS): John A. Moore, D.V.M.

DATE CONTRACT INITIATED: August 15, 1975

CURRENT ANNUAL LEVEL: $300,000

PROJECT DESCRIPTION

OBJECTIVES: To collect, organize, and help disseminate information about reports of testing and evaluation of the teratogenic potential of any environmental agent whether chemical, biological, or physical.

METHODS EMPLOYED: Literature is located by (1) using a teratology profile to search several secondary literature sources; (2) consistently screening forty key journals; (3) personal contact with teratologists; (4) reviewing bibliographies in books and monographs; and (5) using other resources available to ORNL such as the EPA/NIH Structure Search System for chemicals and their library which contains 200,000 bound volumes, 600,000 reports, and 3,500 journals.

MAJOR FINDINGS AND PROPOSED COURSE: Since July 1975 ETIC has built a computer file of approximately 15,000 records and assembled a document library that contains every paper indexed. Each citation has been indexed with all bibliographic information, common and taxonomic name of test object, and Chemical Abstract Service (CAS) Registry Number. Titles may be searched using key words from the title.

The Chemical Agent Registry has been updated to include chemical name fragments and Wiswesser Linear Notations (WLN). As a result chemicals in the ETIC Agent Registry may now be searched by synonym; CAS number; fragment as part of a chemical name; molecular formula; complete structure or fragment structure using Wiswesser Linear Notation codes; structure or structural fragment by diagram; and functional or major chemical components by name (e.g., esters, ethers, epoxides, etc.).

The ETIC file is now one of the subfiles of the National Library of Medicine's TOXLINE (Toxicology Information Online) and is available through a nationwide NLM network of centers at more than 900 universities, medical schools, hospitals, government agencies, and commercial organizations as well as a number of foreign centers.

The ETIC file is also one of the data bases provided by RECON (Remote Console) a remote-access computerized storage and retrieval system located at Oak Ridge National Laboratories and available to all Department of Energy divisions and contractors.

The Chemical Agent Registry has been made a part of the Environmental Protection Agency/National Institutes of Health Substructure Search System and thereby offers still another search capability.

An average of 33 inquiries a month requesting information about over 100 agents and almost every test object are received and processed directly by the ETIC staff. There is no way to determine the number of requests processed through TOXLINE and RECON since they do not monitor searches on those systems.

It is proposed that ETIC will continue to collect and process papers from current and previous years and provide regular updates of the ETIC/TOXLINE subfile and the ETIC/RECON data base. We hope to be able to add author abstracts to the file. The author's address is one new field to be added and is already a part of a number of the other files on TOXLINE. You will be able to identify experts in a particular area by searching with zip codes. Based upon the needs and requirements shown through reviewing search requests, other new files may also be added. Publications of special search subjects will be prepared and made available upon request. A bibliographic listing will be prepared and updated on a regular basis. Primary effort will go into the next major undertaking of ETIC. They will begin the extraction of more detailed information from each reference. This information will then be entered into a Teratology Data Bank.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Because the field of teratology is relatively new and because teratology data on chemical and other agents are widely scattered in the literature, there is an urgent need to bring together data pertaining to this area. To this end, ETIC has organized much of the literature about the teratogenic effect of agents into a computerized system. The extracts to be prepared from each reference and entered into a Teratology Data Bank will make additional information available to users, i.e. dose levels tested, route of administration, vehicle used, number and type of malformations observed, number of animals tested, etc. These systems may be used to minimize unplanned duplication of tests, to aid in determination of the teratogenic status of individual agents, and to establish priorities in the planning of future investigations.

ENVIRONMENTAL CHEMISTRY BRANCH

ENVIRONMENTAL CHEMISTRY BRANCH
Summary Statement

Environmental chemistry involves studies of the chemistry of compounds and their mixtures present in the environment which show undesirable biological or otherwise deleterious effects on living organisms with particular emphasis on xenobiotic, persistent molecules. Such environmental agents can include elements, organic and inorganic compounds, both natural and synthetic. Environmental health chemistry attempts to bring such activities to a focus on potential human hazards of environmental agents, singly and in combination. The major divisions of environmental health sciences which are necessary to define a hazard are environmental epidemiology, toxicology, analysis and chemistry. The increasing importance of chemistry in this overall scheme has been recognized by the elevation of the chemistry programs at NIEHS to Branch level status. The lipophilic organic xenobiotics and, in particular, the halogenated organics are still a major area of attention and research emphasis in the Branch.

SUPPORT ACTIVITIES

Environmental chemistry interfaces environmental analysis, detection and measurement of environmental agents, with environmental toxicology, detection and measurement of various deleterious biological activities of environmental agents. Therefore, chemists are frequently consulted within NIEHS to advise biological scientists in appropriate areas of their research. The support activities of the Chemistry Branch, as expected, fluctuate depending upon the resources required to meet a particular need. Chemistry support requests are increasing in terms of the number and the degree of involvement. The requests generally fall into one of two categories: short term requests such as compound purity determinations and organic synthesis by published procedures, and a variety of long term requests which often require new and improved methodology development such as qualitative and quantitative analysis of complex mixtures and organic synthesis by unpublished procedures. In the latter case, it is difficult to determine the amount of time and effort which will be necessary to complete the task. A trial period may be necessary to demonstrate feasibility within the limitations of the group. Therefore, for obvious reasons these latter requests are carefully evaluated, and we frequently seek to do this work on a collaborative research support basis.

Chemistry support generally involves the use of established known methodologies as opposed to the development of new ones. New methods development will be considered when it appears to be particularly relevant to ongoing programs and is appropriate to the expertise of the group. Special consideration is given to requests that cannot be done outside NIEHS or where clearly we can do it better. In addition, Chemistry provides support in securing procurement and research contracts of a chemical nature. Chemistry support requests are assigned to one of three work groups. These work groups also form the basis for research activities within the Branch; viz. Analytical Biochemistry, Bioorganic Chemistry and Synthesis, and Specialty Instrumentation.

The preparation, determination and characterization of various compounds and compound classes of general interest to the Institute will facilitate the successful elaboration of their chemistry and biochemistry and permit more definitive biological and toxicological studies.

The gas chromatography/mass spectrometry (GC/MS) facility made the largest number (over 2500) of sample runs for analyses. About 25% of these was for routine service request, and the remaining in collaborative support of various biological studies and instrumental and method development research. About 44% of the total samples run were performed using the high resolution mass spectrometer with the data system reflecting a substantial increase in its use and dependence on the more sophisticated system.

High pressure liquid chromatography (HPLC) equipment continues to be maintained by Branch personnel for use by other Institute staff. About 300 analyses were performed through scheduling the use of this equipment.

The nuclear magnetic resonance (NMR) facility handled a number of different samples including a variety of chemical and biological metabolites and custom synthesized chemicals for toxicological testing. In many of these compounds, both ^1H and ^{13}C nuclei using ^1H decoupling and fourier transform (FT) techniques were observed. Our research NMR spectrometer is currently being operated twenty-four hours a day, seven days a week. Most of these samples were generated from projects within the Branch. However, instrument time needed to support other Institute collaborative programs has increased substantially.

New and repeat synthesis of various halogenated hydrocarbons (especially the PCB's) for testing purposes continues to be a major involvement of the synthetic group. Other efforts involve further purification, characterization and identification of compounds available from other sources within and outside the Institute.

A number of other requests were made for analytical service which required specific methods. These ranged from specific analysis for various biological entities such as lipid classes and fatty acids, specific isomer content, metabolites and purity and radiopurity of unlabeled and labeled research chemicals to determination of organic vapors in air and solvent resistance of materials for the Institute safety program.

Chemical management services continue to facilitate the procurement and safe handling of specialty research chemicals during storage, analysis, testing and disposal. These activities are in general support of the newly initiated National Toxicology Program and the mutagenicity testing program in particular.

RESEARCH ACTIVITIES

The nature of the research in the Chemistry Branch is to some degree, predetermined by its support commitment and space limitations. For example, the Chemistry Branch attempts to provide broad areas of science competence rather than indepth and narrow science expertise. However, this requires the availability of diverse instrumentation which consumes space and requires routine maintenance. There are five major areas of research involvement in the Chemistry Branch which are using selected classes of chemical compounds as models and/or special chemical, biochemical and instrumental techniques to develop general methodology of value to environmental health scientists.

Immunochemistry and Immune Functions:

Immunoassay offers advantages of extreme specificity and sensitivity such that detection of hazardous compounds, mutated proteins, residues, etc. at biologically meaningful levels may be feasible with this approach. The radioimmunoassay for chlorinated dibenzo-*p*-dioxins has been completed and a patent application filed. A contract is in negotiation to have this (and other similar) assays validated independently and applied to environmental samples. The immunoassay "fingerprinting" technique for PCBs has proven useful in that it was able to identify the particular Aroclor mixture in a sample of transformer fluid. Conventional techniques failed because of diphenyl ether interference. Antisera have been raised against chlorinated dibenzofuran antigens; one such antiserum shows very high sensitivity while another shows good selectivity for furans over dioxins. The quantitative effects of mixtures of dioxins and furans on the RIA for dioxins have been investigated. There is a synergistic effect. More complete characterization of all of the presently available antisera is needed, but is hindered by the lack of sufficient dioxin and furan standards.

Related studies have indicated that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and the structurally related compound 2,3,7,8-tetrachlorodibenzofuran may exert their immune system effects in mice by direct action on lymphocytic function. These compounds may bind directly to a lymphoid cell inducing lymphotoxicity and eventual loss of normal immune function (cell mediated). This cell is presumably a T-cell. At lower concentrations these compounds may not be lymphotoxic but do effect T-cells probably through a defect in maturation. Attempts are being made to elucidate the maturational defect and detect and isolate lymphocyte and other cell type receptors for such compounds. Studies are continuing to examine the effects of selected environmental agents on the immune system in various laboratory animals. Low level chronic exposure of rodents to polybrominated biphenyls (PBB's) induces immunosuppression only at levels near the toxic dose. *In utero* exposure of mice to PBB has no adverse effects on the immune system.

Mechanism Elucidation:

There is increasing evidence that certain highly toxic halogenated hydrocarbons may have specific binding receptors in biological systems which differ quantitatively in their ability to bind both halogenated and non-halogenated planar molecules. Binding propensity and toxicity may be correlatable. Chemical and biochemical studies are being conducted to provide an understanding of the specific molecular level interactions involved in binding which may in turn permit one to predict, prevent, or reverse them. The guinea pig was used as an extremely sensitive animal model to investigate toxic effects of certain halogenated aromatic hydrocarbons. These compounds include members of the biphenyl, naphthalene, dibenzofuran and dibenzo-p-dioxin classes and other related compounds. Highly toxic members of each of these classes were found with bromine substitution required on the naphthalene nucleus. Test compounds have provided additional evidence that more than two lateral chlorines are required for high toxicity. Structurally important requirements can be broadly classified in terms of molecular symmetry and size. Underlying stereoelectronic requirements include molecular symmetry and permanent dipoles, molecular thickness and planarity (or coplanarity), molecular width and distances between lateral halogens; and sufficient numbers, positions and kinds of halogens.

The hexachlorobiphenyls continue to undergo study as model PCB's to relate structure to mechanism of action. The effects of single oral doses of selected isomers on lipid metabolism in the rat are being studied relative to levels of a number of selected enzyme activities involved in lipid biosynthesis and metabolism as well as to levels of the microsomal drug-metabolizing enzymes and the electrophoretic properties of cytochrome P-448/450. Acceptable methods or modifications of methods have been standardized and will now be applied.

These and related aromatic compounds are known to be metabolized to oxygenated derivatives via reactive intermediary arene oxide metabolites. Such processes can be both toxifying (activating) as well as detoxifying to the biological system. The abundant glutathione S-transferase enzyme system is being studied as a presumed important detoxifying pathway for such compounds. Both regiospecificity and stereospecificity have been demonstrated for the enzymatic conjugation of glutathione with the model compound styrene oxide. This work is being extended to benzo[a]pyrene-4,5-oxide. This work has required the development of unique synthetic, chromatographic, and spectral methods for the preparation, characterization and determination of the metabolites.

Other work includes evaluating the capabilities of various tissue lipases and esterases as early mediators in the interactions of animals with xenobiotics containing ester linkages. These enzymes are also used as models in studies of protein-ligand interactions. A new procedure for the purification of nonspecific lipase has been developed, permitting its preparation on a larger scale than was previously possible. This will permit more detailed study of the mechanism and function of this enzyme system as well as investigation of its synthetic utility. Past studies have led to partial characterization of the enzyme in human plasma responsible for the hydrolyses of phthalate ester plasticizers and has facilitated the pharmacokinetic elaboration of this chemical in man. Plans are underway to use ^{13}C-labeled diethylhexyl phthalate for studies of this plasticizer pharmacokinetics in man in a collaborative effort with the Department of the Army.

In the area of inorganic environmental agents, early work using NMR spectroscopy has indicated that ^{207}Pb NMR may be a very useful probe into the specific binding of lead. Preliminary studies show lead chemical shifts to be dependent on the oxidation state of lead, the coordination number of lead, and the nature of the ligands binding to lead.

Mass Spectral (MS) Methods Development:

Mass spectrometry (in combination with gas chromatography) is still the most sensitive, specific and versatile analytical method available for dealing with complex samples of environmental/biological origins. Negative chemical ionization MS techniques are continuing to be developed for the more sensitive and specific analysis of environmental agents. Two more classes of compounds were studied by this technique, viz. chlorodiphenyl ethers and their hydroxy derivatives, and selected organometallic complexes. The compounds were shown to have characteristic fragmentation sensitive to the nature of the substitution pattern and, in representative cases, to be detectable at lower levels than with conventional positive ion techniques.

Field-free region studies with selected environmental agents using high resolution mass spectrometry with metastable scanning has shown considerable promise in providing specific structural information useful in residue analysis as well as in establishing "chemical appearances" of environmental agents. Computer programs have been developed which permit data system acquisition and processing of ion kinetic energy spectra. Mass-analyzed ion kinetic energy spectra of alkoxide and carboxylate ions have been obtained after collisional excitation and are easily correlated with structure. This approach should complement gas chromatography/mass spectrometry capabilities.

Studies in field desorption mass spectrometry should illuminate the micro-environments important in environmental chemical carcinogenesis. Several adducts and derivatives have been obtained for study as soon as useful emitters for the instrument are prepared.

Molecular orbital calculations have been done to determine minimum energy configurations of neutral molecules and positive and negative ions in support of mass spectral analysis of compounds of environmental interest.

Other Analytical Methods Development:

It is often necessary to develop and refine methodology for the quantitative and qualitative determination of compounds and classes of compounds of general interest to the Institute and specific interest to individual investigators. Recent work has emphasized development and validation of methods for (a) quantitative and reproducible extraction and clean-up of halogenated aromatic compounds from biological materials for subsequent GC-MS or immunoassay, and (b) fortifying samples under conditions yielding equilibration of "spike" with endogenous compound. Classes of compounds studied have included the chlorinated dibenzo-p-dioxins and dibenzofurans, the polychlorinated and polybrominated biphenyls and their related and associated classes such as the chlorinated benzenes, phenols, diphenylethers and biphenyl dimers, some of which are formed under actual use conditions of commercial mixtures. Sample types have included liver, serum, milk, milk substitutes and adipose tissue from rodents, cows, monkeys, and humans. Studies of used fluids such as PCB based transformer and heat exchange (from Yusho oil) fluids have afforded methods for class separation of such compounds in complex mixtures. The analytic methods were developed using gas chromatography, thin-layer and column chromatography, fluorometry, spectrophotometry (IR, UV, Visible), mass spectrometry and isotopic methods. Other special methods were employed where necessary. ^1H and ^{13}C-NMR spectroscopy is also frequently used and was used effectively to characterize toxic fungal metabolites isolated from molded peanuts.

Synthetic Methods Development:

Expertise in synthetic organic chemistry is highly desirable for supporting a variety of research programs at the Institute. Not only are a diverse nature of custom organic chemicals required but they must also be of high purity. Some of these chemicals are also highly toxic (or potentially) and require rather elaborate laboratory design facilities and equipment for maximum compound containment and control. Several chlorinated dibenzofurans have been synthesized in our special glove box laboratory designed for this purpose.

Other studies have involved the development of rather unique synthetic methodologies to permit preparation of specifically labeled compounds and unstable and/or reactive compounds which may represent intermediary metabolites. Methods were developed for specific fluorine labeling of halogenated biphenyls, preparation of biphenyl oxide and diethylstilbestrol metabolites.

Stereospecific syntheses may also be required since optically pure forms may be desired for study such as the chiral synthesis of thromboxane B_2 (active intermediates in prostaglandin metabolism) previously developed. A model system to study the chemical properties of thromboxane A_2 has been designed and has already provided an epimeric analog for study.

A "spin-off" from such efforts has been the development of selective transformations in molecules containing chemically similar groups. This is of great value in synthetic organic chemistry since it expands the choice of starting materials and minimizes the use of separation procedures.

OTHER ACTIVITIES

The Chemistry Branch continues to provide unique analytical chemistry support to the study of environmental contamination problems of imminent public health concern. Through contract analytical chemistry work the Branch continues to support the epidemiological study to evaluate the health effects on infants of specific components of infant foodstuffs, particularly chlorinated pesticide residues and polychlorinated biphenyls (PCBs) in breast milk. This study requires well designed sample collection, analysis and data interpretation schemes. Contractors have essentially completed development and validation studies for analyses in human milk blood (serum) and placenta tissue and will soon initiate analysis of the first sets of mother's samples. A new method was developed for desalting human milk and serum which should permit determination of total organic halide content.

Other studies with human milk attempted to provide unequivocal confirmation of TCDD residues in human milk. The comparative results of these studies along with previously reported and related studies fairly conclusively establishes that the present state-of-the-art will not permit confirmation at below 10 ppt levels in human milk.

The Branch has received and fulfilled requests from several organizations both nationally and internationally to provide analysis of important samples by unique methods developed here. A number of these samples were taken from humans (blood and adipose tissue).

Other contract work is attempting to provide ^{14}C-labeled 2,3,7,8-tetrachlorodibenzofuran for biological study with respect to species differences in adsorption, metabolism, distribution and excretion. The furans are associated highly toxic impurities of the commercial PCB preparations. The desired labeled compound is currently being isolated from the crude reaction mixture of chlorinated furans and should be available for study in pure form in the near future.

Invitations to present lectures, teaching assignments at local universities and nominations to standing committees are accepted without rancor and are usually followed by active participation. The Chemistry Branch is striving hard to establish a rapport with the various pertinent departments of the local universities in order to further enhance its research productivity and provide professional growth. In addition to supporting a continuing seminar program with invited speakers, Branch scientists have also organized specialized workshops/symposia to remain abreast of current methodology and other developments in their field. These included a second local workshop in negative chemical ionization mass spectrometry and two symposia at national meetings; one dealing with the chemist's viewpoint of safe handling of chemical carcinogens, mutagens and teratogens; and the other with the general and conceptually new area of environmental health chemistry.

A new program initiative in bioinorganic chemistry is being considered. A thorough literature survey is being made and reviewed as the first stage of consideration and development of such a program.

The Chemistry Branch remains cognizant of the Institute safety program. A special hazardous chemical containment facility for the Branch has been completed and is operational. The facility now permits a variety of chemical operations involving hazardous chemicals to be conducted with the minimum exposure possible to laboratory personnel. The Branch Office continues to cooperate with the Safety Office in providing chemistry input as needed. The Chief of the Chemistry Branch continues to serve as Chairman of the Safety Committee.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30003-07 ECB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Development of Analytical Methodology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.W. Albro	Research Chemist	ECB NIEHS
OTHER:	J.R. Hass	Research Chemist	ECB NIEHS
	K. Chae	Chemist	ECB NIEHS
	K. Kohli	Visiting Fellow	ECB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This general project area has as its objective, to develop and refine methodology for the quantitative and qualitative determination of compounds and classes of compounds of general interest to the Institute and specific interest to individual investigators. Recent work has emphasized development and validation of methods for (a) quantitative and reproducible extraction and clean-up of halogenated aromatic compounds from biological materials for subsequent GC-MS or immunoassay, and (b) fortifying samples under conditions yielding equilibration of "spike" with endogenous compound.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The analytic methods will generally be developed using gas chromatography, thin-layer and column chromatography, fluorometry, spectrophotometry (IR, UV, Visible), mass spectrometry and isotopic methods. Other special methods will be employed where necessary or as other instrumentation becomes available.

MAJOR FINDINGS AND PROPOSED COURSE: Methods for the extraction and cleanup of halogenated aromatics from liver, serum, milk, milk substitutes and adipose tissue have been refined and extensively validated. Methods for removing inorganic chlorine from milk and serum have been validated. Methods for spiking liver, milk, serum and adipose tissue such that recovery of spike corresponds to recovery of endogenous compound have been worked out and validated. Cleanup procedures suitable for determination of TCDD at the low pg level are being investigated. Chromatographic procedures for the class-fractionation of transformer fluid, and methods for perchlorination of the fractions were developed. Methodologies will continue to be developed as needed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Successful development of the analytical methodology in the areas delineated above will accelerate the successful elaboration of a number of metabolic and degradation studies in progress in the Chemistry Branch as well as be of utility for other studies within the Institute.

PUBLICATIONS

Albro, P.W., and Parker, C.E.: A comparison of the compositions of Aroclor 1242 and Aroclor 1016. J. Chromatogr. 169: 161, 1979.

Albro, P.W.: Problems in analytical methodology. Sample handling, extraction and cleanup. Ann. N.Y. Acad. Sci. (in press).

Kohli, K.K., Albro, P.W., and McKinney, J.D.: Radioisotope dilution assay (RIDA) for the estimation of polychlorinated biphenyls. J. Anal. Toxicol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30012-06 ECB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of Pure Isomers of Hexachlorobiphenyls on Lipid Metabolism and Cell Structure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. Kohli	Visiting Fellow	ECB NIEHS
OTHER:	P. Albro	Research Chemist	ECB NIEHS
	B. Gupta	Veterinary Medical Officer	EBB NIEHS
	H. Mukhtar	Visiting Associate	LP NIEHS
	J. McKinney	Supervisory Research Chemist	ECB NIEHS

COOPERATING UNITS (if any)

Animal Husbandry Section, CMB; and Marine Pharmacology Section, LP

LAB/BRANCH

Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.9	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of single oral doses of 3,3',4,4',5,5'-; 2,2',4,4',5,5'-; and 2,2',3,3',5,5'-hexachlorobiphenyls on lipid metabolism in the rat are being studied relative to levels of activity of fatty acid-CoA ligase, glycerol-3-phosphate acyl transferase, diacylglycerol acyl transferase, diacyl glycerol choline phosphotransferase, lysophosphatidic acid acyl transferase and phosphatidic acid phosphohydrolase. In addition, the effects of the above PCBs on microsomal drug-metabolizing enzymes and on the electrophoretic properties of cytochrome P-448/450 are being examined. The general goal of these studies is to relate PCB structure to some of its mechanisms of action.

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard lipid chemistry analytical methods, oral feeding of PCBs in corn oil by gavage, routine enzyme assays, histologic examination.

MAJOR FINDINGS AND PROPOSED COURSE: Since the previous report, efforts have been directed toward selecting and standardizing assays for the enzymes involved in lipid biosynthesis as well as electrophoretic conditions suitable for resolution of P-448/450 subfractions. We have observed that several of the enzyme assays in the literature are unreliable even to the point of being measures of artifacts rather than enzyme products. However, acceptable methods or modifications of methods have now been standardized, so the actual study is ready to begin.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies, and related studies of structure-activity relationships for the family of PCB compounds will contribute to our ability to predict the hazards of previously untested PCB-like compounds. This is desirable because of the ubiquitous nature of PCB pollution of the environment and the variety of PCB isomers and related compounds involved.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30015-05 ECB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Studies in Chemical Ionization Mass Spectrometry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. R. Hass	Research Chemist	ECB NIEHS
OTHER:	None		

COOPERATING UNITS (if any)
M.M. Bursey, Department of Chemistry, University of North Carolina, Chapel Hill, NC

LAB/BRANCH
Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The negative ion chemical ionization mass spectra of typical chlorodiphenyl ethers, hydroxychlorodiphenyl ethers and selected organometallic complexes have been obtained and interpreted.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard gas chromatography/mass spectrometry techniques, negative ion chemical ionization mass spectrometry.

MAJOR FINDINGS AND PROPOSED COURSE: Three classes of compounds were studied by negative ion chemical ionization mass spectrometry: chlorodiphenyl ethers, hydroxychlorodiphenyl ethers, and selected organometallic complexes.

The compounds were shown to have characteristic fragmentations sensitive to the nature of the substitution pattern and, in representative cases, to be detectable at lower limits than with conventional positive ion chemical ionization mass spectrometry. Correlations of substitution pattern with specific reactions observed in negative ion chemical ionization mass spectrometry were made for these compounds which will act as guides to understanding the specificity of certain losses and ion-molecule reactions. The negative ion chemical ionization mass spectra of these compounds were shown to depend on the site of substitution and other structural features; several unprecedented types of ion-molecule reactions were observed.

This project will be extended by the study of the CI mass spectra of other environmental hazards.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Negative ion chemical ionization mass spectrometry was shown to permit analysis of several classes of environmental contaminants at lower levels than previously possible. For others, a better understanding of the interpretation of the mass spectra will lead to better methods of structural identification of organic contaminants at the ppb level. Chemical ionization techniques have provided information complementary to that obtained using electron impact ionization methods. The methods employed in this project allows one to gather further information concerning the nature of an unknown sample.

PUBLICATIONS

Busch, K.L., Bursey, M.M., Hass, J.R., and Sovocool, G.W.: Comparison of five ionization methods for producing mass spectra of typical organophosphorus pesticides. Appl. Spectrosc. 32: 388-299, 1978.

Busch, K.L., Hass, J.R., and Bursey, M.M.: The gas enhanced negative ion mass spectra of polychloroanisoles. Org. Mass Spectrom. 13: 604-607, 1978.

Hass, J.R., Friesen, M.D. and Hoffman, M.K.: The mass spectrometry of polychlorinated dibenzo-p-dioxins. Org. Mass Spectrom. 14: 9-16, 1979.

Busch, K.L., Norstrom, A., Bursey, M.M. and Hass, J.R.: Methane-oxygen enhanced negative ion mass spectra of polychlorinated diphenyl ethers. Biomed. Mass Spectrom. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30018-06 ECB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Ester Hydrolases at the Environmental Interface

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. W. Albro	Research Chemist	ECB NIEHS
OTHER:	None		

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to evaluate the capabilities of various tissue lipases and esterases as early mediators in the interactions of animals with xenobiotics containing ester linkages. These enzymes are also used as models in studies of protein-ligand interactions. A new procedure for the purification of nonspecific lipase has been developed, permitting its preparation on a larger scale than was previously possible. Its interaction with cofactors and substrates will be studied by circular dichroism and fluorescence spectroscopy.

PROJECT DESCRIPTION

METHODS EMPLOYED: Most studies will involve the rat, but significant findings will be checked in mice, hamsters, guinea pigs and rabbits. Lung, gastrointestinal, and skin enzymes will be studied, using techniques generally described previously (Biochim. Biophys. Acta, 360: 380, 1973). Test substances will initially include: (1) esters of 2,4-D and 2,4,5-T, (2) phthalate plasticizers, (3) detergents such as Tween 20, Tween 80, (4) aromatic rubrefacients such as methyl paraben, menthyl salicylate, (5) volatile solvents such as ethyl acetate, butyl acetate, (6) carbamates.

MAJOR FINDINGS AND PROPOSED COURSE: Planned studies of protein-lipid interactions using nonspecific lipase from rat pancreas were delayed since no bids were received on our request for a large-scale purification of the enzyme. These studies have barely begun, as a result of our having developed a new purification method enabling us to purify larger amounts of enzyme in-house.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Relatively little is known about non-specific lipase activity, since the enzyme(s) has(have) never previously been purified. Although a great deal is known about lung esterases, very little is known about lung lipases (hydrolases for insoluble esters). Virtually nothing is known about ester hydrolases in skin. These enzymes may either protect against some hazardous compounds by converting them to less easily absorbed, more polar materials, or increase the hazard of other compounds when the acid (or alcohol) is more toxic than the ester. A second anticipated result of this project should be a better understanding of how enzymes in general are able to bind to and react with water-insoluble compounds.

PUBLICATIONS

Albro, P.W., and Corbett, J.T.: Distribution of di- and mono-(2-ethylhexyl) phthalate in human plasma. Transfusion 18: 750, 1978.

Peck, C.C., Odom, D.G., Friedman, H.I., Albro, P.W., Hass, J.R., Brady, J.T., and Jess, D.A.: Di-2-ethylhexyl phthalate (DEHP) and mono-2-ethylhexyl phthalate (MEHP) accumulation in whole blood and red cell concentrates. Transfusion 18, 750, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30020-08 ECB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Transport and Metabolism of Phthalate Esters

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. W. Albro	Research Chemist	ECB NIEHS
OTHER:	J. R. Hass	Research Chemist	ECB NIEHS

COOPERATING UNITS (if any)

C. C. Peck, M.D., LTC, MC, LAIR

LAB/BRANCH
Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.0	PROFESSIONAL: 0.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

CID processes have been used to confirm the identification of ten metabolites of diethylhexyl phthalate (DEHP), which have been isolated in small quantities as reference standards for metabolism studies. Plans are underway (at LAIR) to use ^{13}C-labeled DEHP for studies of plasticizer pharmacokinetics in man. The role of NIEHS will be solely to analyze plasma and urine samples provided by LAIR. No work on this project was done this year due to the delay in LAIR obtaining FDA approval for use of human subjects.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Chromatography, spectrophotometry, mass spectrometry, isotopic methods, standard enzymology techniques.

MAJOR FINDINGS AND PROPOSED COURSE: The previously isolated (rat) metabolites of DEHP have been unequivocally identified by mass spec CID methods. The proposed study of ^{13}C-phthalate pharmacokinetics in man is delayed while the necessary permission to use human subjects is obtained by LAIR.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The phthalic acid esters are important industrial chemicals employed with extensive utility, primarily as plasticizers. Recent evidence of their migration into human tissues as well as their increasing occurrence in the ecology have been cited. Knowledge of the consequences of chronic or subacute ingestion, absorption and/or inhalation is essential for assessing the parameters of potential hazard of this environmental agent.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30034-04 ECB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Chemistry of Aromatic Compounds and Their Environmental Transformation Products

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. A. Levy	Research Chemist	ECB NIEHS
OTHER:	None		

COOPERATING UNITS (if any)
Environmental Biology Branch, NIEHS
Laboratory of Environmental Toxicology, NIEHS

LAB/BRANCH
Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 1.25	PROFESSIONAL: 1.0	OTHER: 0.25

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The development of rational synthetic routes to halogenated biphenyls, biphenyl oxide, polynuclear aromatic hydrocarbons and diethylstilbestrol metabolites have been investigated. Models appropriate to the study of the chemical and physical properties of these classes of compounds as potential persistent environmental agents have been prepared.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Synthetic techniques, organometallic reagents and catalysis, high pressure reactions, photochemical processes, functional group transformations. Mass spectroscopy, other spectroscopic methods (IR, NMR), chromatography (column, glc, hplc).

MAJOR FINDINGS AND PROPOSED COURSE: The synthesis of 3,4-biphenyl oxide, the presumed intermediate in the metabolism of biphenyl, has been accomplished. The unstable nature of this compound has so far precluded its isolation. However, its existence has been inforced by the presence of the appropriate phenolic products which can only arise *via* isomerization of the arene oxide.

The synthesis of 3,5,3',5'-tetraflouro-4,4'-dichlorobiphenyl was carried out in order to probe those factors responsible for the toxicity of the chlorinated biphenyls.

New approaches to the synthesis of polynuclear aromatic hydrocarbons (PAH) have been successfully investigated. Using a concept previously exploited for the synthesis of substituted naphthalenes, the cycloaddition of *ortho*-xylylene with appropriate dieneophiles has led to PAH's as well as derivatives with potential for elaboration to more complex structures heretofore not available or only obtainable with great effort.

A number of presumed and actual metabolites of diethylstilbestrol (DES) were prepared for a study on the mechanism of action of this synthetic estrogen. This work has been carried out in collaboration with the transplacental toxicology group in the laboratory of environmental toxicology.

It is proposed to continue the development of new synthetic approaches to polynuclear aromatic hydrocarbons and their metabolites such as arene oxides and hydroxylated derivatives. It is also proposed to continue the collaboration with transplacental toxicology group into the mechanism of hormonal activity of DES. The preparation of appropriate models of the above mentioned classes of compounds will be carried out, as time permits, to more fully explore their chemical and spectral properties.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Polychlorinated biphenyls, polynuclear aromatic hydrocarbons and diethylstilbestrol are recognized to be of major environmental importance due to their widespread ecological distribution and, in some cases, their concentration in the food chain. The availability of the unique isomers of these classes of compounds and their probable metabolites would permit critical biological and toxicological studies. Synthetic availability of appropriate model compounds allows further examination of the mechanisms of their biological activity.

PUBLICATIONS

McLachlan, J.A., Baucom, K., Korack, K.S., Levy, L., and Metzler, M.: A novel fluorinated derivative of diethylstilbestrol. *Steroids* (in press).

Levy, L.A.: Stereochemistry of the transition state of hydrogen abstraction by vinyl radicals. *J. Chem. Soc. Chem. Comm.* 574, 1978.

Levy, L.A.: Synthesis of unsymmetrical biphenyls *via* aryl substituted 1,4-cyclohexadienes. *J. Org. Chem.* 43: 3062, 1978.

Hass, J.R., Bursey, M.M., Levy, L.A., and Harvan, D.J.: Structural correlations with kinetic energy release during loss of chlorine from nineteen selected chlorinated biphenyls. *Org. Mass Spectrom.* (in press).

Goldstein, J.A., Linki, P.C., Levy, L.A., McKinney, J.D., Gupta, B.N., and Moore, J.A.: A comparison of the effects of a commercial polybrominated biphenyl mixture, 2,4,5,2',4',5'-hexabromobiphenyl and a brominated naphthalene of hepatic mixed function oxidases. *Biochem. Pharmacol.* (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30035-04 ECB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Chemical Techniques and Spectroscopic Methods

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. D. McKinney	Supervisory Research Chemist	ECB NIEHS
	P. A. Albro	Research Chemist	ECB NIEHS
	J. R. Hass	Research Chemist	ECB NIEHS
	R. H. Cox	Research Chemist	ECB NIEHS
OTHER:	K. C. Chae	Chemist	ECB NIEHS
	L. A. Levy	Research Chemist	ECB NIEHS
	C. E. Parker	Research Chemist	ECB NIEHS
	D. B. Walters	Technical Programs Coordinator	ECB NIEHS

COOPERATING UNITS (if any)

NCI Chemical Repository.
Others as needed.

LAB/BRANCH

Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.1	1.6	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This general project has as its objective to employ chemical techniques, both analytical and synthetic, and spectroscopic methods, in particular gas chromatography/mass spectrometry (GC/MS), nuclear magnetic resonance spectroscopy (NMR) and high pressure liquid chromatography (HPLC), to the preparation, determination and characterization of compounds and classes of compounds of general interest to the Institute and specific interest to individual investigators. In general compounds covered by this study will include those for which adequate techniques and methods are already available or can be adapted. Chemical management services are also performed in support of various research programs.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: The analytical methods emphasized in this study are GC/MS, NMR or fourier transform NMR and HPLC. A variety of synthetic techniques are employed. Other special methods will be employed where necessary or as other instrumentation becomes available.

MAJOR FINDINGS AND PROPOSED COURSE: The GC/MS facility made the largest number (over 2500) of sample runs for analyses. About 25% of these was for routine service request, and the remaining in collaborative support of various biological studies and instrumental and method development research. About 44% of the total samples run were performed using the high resolution mass spectrometer with the data system reflecting a substantial increase in its use and dependence on the more sophisticated system.

High pressure liquid chromatography (HPLC) equipment continues to be maintained by Branch personnel for use by other Institute staff. About 300 analyses were performed through scheduling the use of this equipment.

The NMR facility handled a number of different samples including a variety of chemical and biological metabolites and custom synthesized chemicals for toxicological testing. In many of these compounds, both ^{1}H and ^{13}C nuclei using ^{1}H decoupling and fourier transform (FT) techniques were observed. Our research NMR spectrometer is currently being operated twenty-four hours a day, seven days a week. Most of these samples were generated from projects within the Branch. However, instrument time needed to support other Institute collaborative programs has increased substantially.

New and repeat synthesis of various halogenated hydrocarbons (especially the PCB's) for testing purposes continues to be a major involvement of the synthetic group. Other efforts involve further purification, characterization and identification of compounds available from other sources within and outside the Institute.

A number of other requests were made for analytical service which required specific methods. These ranged from specific analysis for various biological entities such as lipid classes and fatty acids, specific isomer content, metabolites and purity and radiopurity of unlabeled and labeled research chemicals to determination of organic vapors in air and solvent resistance of materials for the Institute safety program.

Chemical management services continue to facilitate the procurement and safe handling of specialty research chemicals during storage, analysis, testing and disposal.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The preparation, determination and characterization of various compounds and compound classes of general interest to the Institute will facilitate the successful elaboration of their chemistry and biochemistry and permit more definitive biological and toxicological studies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30041-04 ECB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Immunochemistry of Hydrophobic Haptens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. W. Albro	Research Chemist	ECB NIEHS
OTHER:	M. I. Luster	Senior Staff Fellow	ECB NIEHS
	K. Chae	Chemist	ECB NIEHS
	A. Chaudhary	Visiting Fellow	ECB NIEHS
	J. D. McKinney	Supervisory Research Chemist	ECB NIEHS

COOPERATING UNITS (if any)

Animal Husbandry Section, CMB

LAB/BRANCH

Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 2.3	PROFESSIONAL: 1.2	OTHER: 1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A radioimmunoassay for chlorinated dibenzodioxins has been developed. One version of the assay responds to between 20 and 200 pg of dioxin, but is subject to interference. Another version responds to between 90 pg and 10 ng of dioxin. An adequate assay for mono-2-ethylhexyl phthalate in plasma has been completed. Several antisera to specific PCB isomers have been raised and characterized. Antisera which distinguish chlorinated dibenzofurans from chlorinated dibenzo-p-dioxins have been raised and are being characterized.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Synthetic techniques: condensation reactions, amination, reduction, mixed ester formation, active ester formation, convergent synthesis, spectroscopic and chromatographic characterization. Conjugation techniques: diazo coupling, mixed anhydride acylation, active ester acylation. Characterization of conjugates by UV spectroscopy, gel filtration, amino acid analysis, NMR spectrometry, and chemical assay of functional groups. Antibody production methods: standard procedures, with adjuvant, in rabbits. Antibody assay methods: double immunodiffusion, fluorescent antigen, radioimmunoassay.

MAJOR FINDINGS AND PROPOSED COURSE: The radioimmunoassay for chlorinated dibenzo-p-dioxins has been completed and a patent application filed. A contract is in negotiation to have this (and other similar) assays validated independently and applied to environmental samples. The immunoassay "fingerprinting" technique for PCBs has proven useful in that it was able to identify the particular Aroclor mixture in a sample of transformer fluid. Conventional techniques failed because of diphenyl ether interference. Antisera have been raised against chlorinated dibenzofuran antigens; one such antiserum shows very high sensitivity while another shows good selectivity for furans over dioxins. The quantitative effects of mixtures of dioxins and furans on the RIA for dioxins have been investigated. There is a synergistic effect. More complete characterization of all of the presently available antisera is needed, but is hindered by the lack of sufficient dioxin and furan standards.

The following compounds have been synthesized in the present reporting period: 2,7,8-Trichlorodibenzofuran; 2,7,8-trichloro-4-aminodibenzofuran; 4,7,8-trichloro-2-aminodibenzofuran; 2,6,9-trichloro-4-aminodibenzofuran; BSA and thyroglobulin conjugates of the aminofurans; p-hydroxyphenyl propionic acid amide of 2,7,8-trichloro-1-aminodibenzo-p-dioxin.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Immunoassay offers advantages of extreme specificity and sensitivity such that detection of hazardous compounds, mutated proteins, residues, etc. at biologically meaningful levels may be feasible with this approach. Most hapten-protein conjugates synthesized in the past have not been well characterized or reproducible, weaknesses hopefully to be overcome in the present project. Chlorinated diphenyl ether derivatives (dibenzodioxins and dibenzofurans) are currently being emphasized since some are exquisitely toxic and may be widespread environmental contaminants.

PUBLICATIONS

Luster, M.I., Albro, P.W., Chae, K., and Clark, G.: Radioimmunoassay for MEHP in unextracted plasma and serum. Clin. Chem. 24: 429, 1978.

Luster, M.I., Albro, P.W., Faith, R.E., and Lawson, L.D.: Inability of passive antibodies to reverse the effects of dioxin toxicity. Chemosphere 1: 29, 1978.

Chaudhary, S.K., and Albro, P.W.: A convenient method for preparation of 2-amino-3',4,4',5-tetrachlorobiphenyl. Org. Prep. Proc. Intern. 10: 46, 1978.

Albro, P.W., Luster, M.I., Chae, K., Chaudhary, S.K., Clark, G., Lawson, L.D., Corbett, J.T., and McKinney, J.D.: A radioimmunoassay for chlorinated dibenzo-p-dioxins. Toxicol. Appl. Pharmacol. (in press).

Luster, M.I., Albro, P.W., Clark, G., Chae, K., Chaudhary, S.K., Lawson, L.D., Corbett, J.T., and McKinney, J.D.: Production and characterization of antisera specific for chlorinated biphenyl species. Initiation of a radioimmunoassay for Aroclors^{R}. Toxicol. Appl. Pharmacol. (in press).

Chae, K., and Albro, P.W.: Synthesis and characterization of amino-mono(2-ethylhexyl) phthalate. Org. Prep. Proc. Intern. 11: 1, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30050-03 ECB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Chemical and Enzymatic Conjugation of Glutathione with Arene Oxides and Epoxides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. D. McKinney	Supervisory Research Chemist	ECB NIEHS
OTHER:	R. H. Cox	Research Chemist	ECB NIEHS
	O. Hernandez	Visiting Associate	ECB NIEHS
	B. Smith	Staff Fellow	LP NIEHS
	J. Bend	Visiting Scientist	LP NIEHS
	B. Yagen	Visiting Scientist	ECB NIEHS

COOPERATING UNITS (if any)
Marine Pharmacology Section, LP, NIEHS, and Midwest Research Institute (Procurement Contract with NCI, No. N01-CP-33387. Supplemented by NIEHS).

LAB/BRANCH
Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.9	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Nuclear magnetic resonance (NMR) spectroscopy is being used to investigate the chemical conjugation of specific benzo[a]pyrene oxides and other arene oxides to glutathione, enzymatically and non-enzymatically. The regiospecificity and stereospecificity of the reaction will be determined. Mechanistic elaboration of the binding process is important in understanding, preventing, and predicting the mechanism(s) of action of such compounds. A high pressure liquid chromatography (HPLC) system was developed to allow analysis of mixtures of glutathione conjugates and to follow their metabolic degradation through several steps down to mercapturic acids.

PROJECT DESCRIPTION

METHODS EMPLOYED: Fourier transform ^{13}C and ^{1}H nuclear magnetic resonance (NMR) spectroscopy; high pressure liquid chromatography (HPLC); organic syntheses, *in vitro* biological experiments.

MAJOR FINDINGS AND PROPOSED COURSE: Careful ^{13}C NMR analysis of the conjugates of styrene oxide with glutathione has revealed significant differences in the ratio of positional isomers formed upon opening of the oxarane ring by the thiol nucleophile. The enzymatic reaction shows large (90+%) preference of attack at the benzylic carbon of styrene oxide while the chemical reaction produces 60% of the benzylic thioether isomer. Both chemical and enzymatic conjugation of 1,2,3,4-tetrahydronaphthalene oxide produces only (99+%) the benzylic thio isomer.

Re-examination of the HPLC separation previously achieved for the styrene oxide conjugates revealed that the two signals observed correspond to the two diastereoisomers of the predominant benzylic thioether with the diastereoisomers of the minor primary thioether overlapping with the second eluting peak from the HPLC system. This problem was circumvented by synthesizing both enonthiomeric forms of optically pure styrene oxide. The positional isomers derived from levorotating styrene oxide separate on HPLC thus providing an analytical method for assaying glutathione-S-transferase activity, positional preferences, relative rates of formation and excretion. The magnitude of this finding will become clear as further metabolic studies are undertaken.

^{13}C NMR spectra of the hydroxy isomers of benzo[a]pyrene (BP) and the BP diols have been recorded and fully analyzed.

Future studies include the synthesis of radiolabeled, ^{3}H or ^{14}C, optically pure levo styrene oxide and the use of this substrate for kinetic and mechanistic studies. The conjugation of glutathione with additional oxides including benzo[a]pyrene-4,5-oxide will be examined to determine the preferred position of attack on the arene oxide and the stereospecificity of the addition product. NMR spectra of the products will be recorded for identification and reference purposes.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Certain arene oxides are known to bind covalently to nucleic acids, and they have been implicated as carcinogens and mutagens which can be derived from the parent compounds. The abundant detoxifying glutathione S-transferase enzyme system is also likely to covalently bind these oxides. Such mechanistic elaboration of the process will aid in understanding, preventing, and predicting the mechanism(s) of action of these compounds.

PUBLICATIONS

Hernandez, O., Cox, R.H., Smith, B., Bend, J.R., and McKinney, J.D.: Structural properties and synthesis of glutathione conjugates of oxides of

styrene and benzo[a]pyrene. Symposium on Polynuclear Aromatic Hydrocarbons, Columbus, Ohio, October, 1978. (abstract)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30051-03 ECB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Characterization of Specific Binding Modes of Organics and Inorganics: The Toxic Polyhalogenated Aromatic Hydrocarbons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.D. McKinney	Supervisory Research Chemist	ECB NIEHS
OTHER:	P. Singh	Research Chemist	ECB NIEHS
	L.A. Levy	Research Chemist	ECB NIEHS
	E.E. McConnell	Veterinary Pathologist	EBB NIEHS
	J.A. Goldstein	Pharmacologist	EBB NIEHS

COOPERATING UNITS (if any)
Comparative Biology Section

LAB/BRANCH
Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The exact molecular structures of some highly toxic polyhalogenated aromatic hydrocarbons have been obtained by X-ray crystallographic measurements or estimated based on postulated structures. The requirements of molecular symmetry and size as determined by the number, kind, and positions of halogens, planarity, interatomic distances and overall stereoelectronics suggest that a specific biological receptor may be involved which could account for the common toxic pattern. The guinea pig will continue to be used as a screening animal model in providing direction for further biological study of related compounds. A possible relationship of this toxicity and carcinogenicity and cocarcinogenicity in ubiquitous polynuclear aromatic hydrocarbons is emerging.

POSITION DESCRIPTION

METHODS EMPLOYED: Synthetic methods along with X-ray crystallography with associated equipment and techniques was used primarily in this phase of the work. Variable temperature high resolution multi nuclei nuclear magnetic resonance (NMR) spectroscopy using specifically labeled (^{13}C, ^{19}F, ^{2}H, etc.) compounds where possible for studying complex molecular interactions is also used when solubility is not a problem. Isolation and characterization of specific binding site(s) in body tissue and fluid using standard biochemical methods.

MAJOR FINDINGS AND PROPOSED COURSE: The guinea pig was used as an extremely sensitive animal model to investigate toxic effects of certain halogenated aromatic hydrocarbons. These compounds include members of the biphenyl, naphthalene, dibenzofuran and dibenzo-p-dioxin classes and other related compounds. Highly toxic members of each of these classes were found with bromine substitution required on the naphthalene nucleus. No unusual toxicity was observed for 3,3',5,5'-tetrafluoro-4,4'-dichlorobiphenyl. This compound is isosteric with 4,4'-dichlorobiphenyl but isoelectronic with the toxic biphenyl 3,3',4,4',5,5'-hexachlorobiphenyl. Testing of the compound provides additional evidence that more than two lateral chlorines are required for high toxicity. Structurally important requirements can be broadly classified in terms of molecular symmetry and size. Underlying stereoelectronic requirements include molecular symmetry and permanent dipoles, molecular thickness and planarity (or coplanarity), molecular width and distances between lateral halogens, and sufficient numbers, positions and kinds of halogens. Although differences in absorption, distribution and metabolism are possible explanations for differences in toxicity, the major difference is thought to be relative binding affinity for a specific biological receptor. Structural requirements important in carcinogenicity of the ubiquitous polynuclear aromatic hydrocarbons are similar in many ways to those found to be important for this toxicity. The possible relationship of this toxicity and carcinogenicity and cocarcinogenicity will be further investigated. The guinea pig will continue to be used as a screening animal model in providing direction for further biological study of related compounds.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There is increasing evidence that certain highly toxic halogenated hydrocarbons may have specific binding receptors in biological systems which differ quantitatively in their ability to bind both halogenated and non-halogenated planar molecules. Binding propensity and toxicity may be correlatable. An understanding of the specific molecular level interactions involved in binding may permit one to predict, prevent, or reverse them.

PUBLICATIONS

Singh, P. and McKinney, J.D.: Crystal and molecular structure of 2,2',4,4',6,6'-hexachlorobiphenyl. Acta Cryst. B35: 259-262, 1979.

McKinney, J.D., Singh, P., Levy, L. and Walker, M.: High toxicity and cocarcinogenic potential of certain halogenated aromatic hydrocarbons. In Walters, D.B. (Ed.): Safe Handling of Chemical Carcinogens, Mutagens, and Teratogens: Chemist's Viewpoint. Ann Arbor, Michigan, Ann Arbor Science Publishing Co., 1979 (in press).

Kohli, K., Mukhtar, H., Bend, J.R., Albro, P.W. and McKinney, J.D.: Biochemical effects of pure isomers of hexachlorobiphenyl: hepatic microsomal epoxide hydrase and cytosolic glutathione S-transferase activities in the rat. Biochem. Pharmacol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30052-03 ECB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
^{13}C-Nuclear Magnetic Resonance Study of Chlorinated Polycyclodiene Pesticides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. D. McKinney	Supervisory Research Chemist	ECB NIEHS
	R. H. Cox	Research Chemist	ECB NIEHS
OTHER:	None		

COOPERATING UNITS (if any)
None

LAB/BRANCH
Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

^{13}C-Nuclear Magnetic Resonance (NMR) is being used to characterize several chlorinated polycyclodiene pesticides and some of their phototransformation and potential metabolic products. ^{13}C-NMR was shown to be superior to proton NMR for identification of these compounds and elucidation of their peculiar electronic interactions which underlie their biological activity.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Fourier transform ^{13}C nuclear magnetic resonance (NMR) spectroscopy and shift and relaxation reagents.

MAJOR FINDINGS AND PROPOSED COURSE: The ^{13}C NMR spectra of several chlorinated polycyclodiene pesticides including aldrin, dieldrin, endrin, and heptachlor, and twenty three of their phototransformation and potential metabolic products (derived from synthesis), have been obtained and interpreted. ^{13}C-NMR was shown to be superior to proton NMR for the identification of these compounds and will aid in elucidating the peculiar electronic interactions in these polycyclic systems which underlie their biological activity. This study is being terminated.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The ultimate fate of the chlorinated polycyclodiene pesticides in the environment continues to be investigated. Therefore, more difinitive techniques to identify their various possible transformation products are needed. Furthermore, it is likely that the biological activity associated with many of these compounds is a function of their peculiar electronic interactions, intra- and intermolecularly.

PUBLICATIONS

Cox, R.H. and McKinney, J.D.: Carbon-13 NMR studies of some chlorinated polycyclodiene pesticides. Org. Mag. Res. 11: 541, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30058-02 ECB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Synthesis of Thromboxanes and Thromboxane Analogs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	O. Hernandez	Visiting Associate	ECB NIEHS
OTHER:	S. Chaudhary	Visiting Fellow	ECB NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Thromboxanes are new kinds of metabolites derived from the prostaglandin endoperoxides PGE_2 and PGH_2. Research seeking to understand the basis for the potent biological activity displayed by these compounds is limited by the lack of availability of samples. The purpose of this work is (1) to design a practical synthesis for the preparation of the title compounds and (2) to study the chemical and biological properties of thromboxanes.

PROJECT DESCRIPTION

METHODS EMPLOYED: Selective functionalization of hydroxyl groups, high pressure liquid chromatography, chemical ionization mass spectrometry, proton magnetic resonance spectroscopy.

MAJOR FINDINGS AND PROPOSED COURSE: A model system to study the chemical properties of Thromboxane A_2 (TXA_2) has been designed. The synthetic sequence has provided an epimeric TXA_2 analog and work is underway to generate an analog with the natural stereochemistry. The synthetic procedure offers potential for the synthesis of sulfur and nitrogen TXA_2 type compounds, both of which analogs have been shown to have interesting biological properties in the prostaglandin series.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Thromboxanes, and in particular thromboxane A_2, are known to play a decisive role in several critical systems of the human body. The mechanism by which this biological activity is exerted, remains unclear. A detailed study of the chemical and biological properties of thromboxanes is of great significance towards the understanding of the physiological role of thromboxanes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30059-02 ECB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Development of New Synthetic Procedures and Reactions.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	O. Hernandez	Visiting Associate	ECB NIEHS
OTHER:	S. Chaudhary	Visiting Fellow	ECB NIEHS
	R. Cox	Research Chemist	ECB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION

NIH, NIEHS, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The ability to effect selective transformations in molecules containing chemically similar groups is of great value in synthetic organic chemistry since it expands the choice of starting materials and minimizes the use of separation procedures. The purpose of this work is to develop methods which allow specific derivatization of primary or secondary alcohols in molecules containing both functionalities. Expertise in synthetic organic chemistry is reflected in support and collaborative activities with other groups in the Institute.

PROJECT DESCRIPTION

METHODS EMPLOYED: Carbon magnetic resonance, proton magnetic resonance spectroscopy and the usual synthetic apparatus and equipment.

MAJOR FINDINGS AND PROPOSED COURSE: The proposed mechanism has been validated in the case of the triphenylmethylation reaction by isolation of a stable N-tritylpyridium salt. This novel compound which is formed readily from triphenylmethylchloride and 4-dimethylaminopyridine at room temperature, has been shown to transfer the triphenylmethyl moiety to primary alcohols with remarkable selectivity. The synthesis and reactivity of related N-pyridinium salts will be further explored because of the great synthetic potential of these transfer reactions.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An active research program on organic synthesis contributes to maintain a high level of expertise on this important discipline in the Chemistry Branch. This expertise is reflected in support and collaborative activities with other groups in the Institute. As an example of this function, a chemical synthesis of thromboxane B_2 was based on the selective functionalization of the hydroxyl groups in glucose.

PUBLICATIONS

Chaudhary, S.K., and Hernandez, O.: A simplified procedure for the preparation of triphenylmethylethers. Tetrahedron Lett. 95-98, 1979.

Chaudhary, S.K. and Hernandez, O.: 4-Dimethylaminopyridine: An efficient and selective catalyst for the silylation of alcohols. Tetrahedron Lett. 99-102, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30060-02 ECB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Structure of Fungal Metabolites

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.H. Cox	Research Chemist	ECB NIEHS
OTHER:	O. Hernandez	Visiting Associate	ECB NIEHS

COOPERATING UNITS (if any)

National Peanut Laboratory, Dawson, Georgia
U.S.D.A., Georgia-South Carolina Area, Tifton, Georgia

LAB/BRANCH
Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.15	0.15	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

^{1}H- and ^{13}C-Nuclear Magnetic Resonance (NMR) is being used to characterize toxic fungal metabolites. New compounds whose structures were determined include two biologically active norditerpenoid ditaclones (trivial names wentilactone A and wentilactone B) from Aspergillus wentii; bis-8,8'(7-hydroxy-4-methoxy-5-methylcoumarin), trivial name orlandin, from Aspergillus niger; 3,7-dimethyl-8-hydroxy-6-methoxyisochroman from Penicillium steckii; aflatoxicol A and B, biological metabolites of aflatoxin B$_1$; and 6-ethyl-5-hydroxy-3-hydroxymethyl-5,6,7,8-tetrahydrochromone.

PROJECT DESCRIPTION

METHODS EMPLOYED: Fourier transform ^1H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy.

MAJOR FINDINGS AND PROPOSED COURSE: A toxigenic fungus identified as Aspergillus wentii was isolated from peanuts which had molded during storage. This fungus produced two new biologically active norditerpenoid dilactones. The structures of the metabolites were determined by spectroscopic means and are related to the podolactones. They were given the trivial names wentilactone A and B. Wentilactone A had an LD_{50} of 7.0 mg/kg when dosed orally to 1-day-old chickens. Both metabolites exhibited plant-growth regulating properties.

A new metabolite, bis-8,8'-(7-hydroxy-4-methoxy-5-methylcoumarin), trivial name orlandin, was isolated from Aspergillus niger found growing in a citrus orchard. Although it is non-toxic to day-old cockerels, it is a significant plant growth inhibitor.

A new toxic metabolite isolated from Penicillium steckii has been identified as 3,7-dimethyl-8-hydroxy-6-methoxyisochroman. The median lethal dose of the toxin in day-old cockerels was 800 mg/kg.

The stereochemistry of aflatoxicol A and B, two biological metabolites of aflatoxin B_1, have been determined by X-ray diffraction techniques. The configuration of C_3 in aflatoxicol B was found to be R, with the orientation of the OH group on C_3 being trans to the A-B ring junction.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Molds found growing on cereal crops in many instances produce toxic fungal metabolites that may possibly ultimately end up in the human food chain (i.e. the aflatoxins). Therefore, identification of these metabolites and screening for biological activity are needed. Furthermore, it is likely that some of the metabolites may possess biological activity beneficial to man.

PUBLICATIONS

Cutler, H.G., LeFiles, J.H., Crumley, F.G., and Cox, R.H.: Hydroxyterphenyllin: A novel fungal metabolite with plant growth-inhibiting properties. J. Agric. Food Chem. 26: 632, 1978.

Newton, M.G., Pautaleo, N.S., Churchill, F., and Cox, R.H.: The stereochemistry of Aflatoxicol B_1. J. Agric. Food Chem. (in press).

Cox, R.H., Hernandez, O., Dorner, J.W., Cole, R.J., and Fennell, D.I.: A new isochroman mycotoxin isolated from Penicillium steckii. J. Agric. Food Chem. (in press).

Cutler, H.G., Crumley, F.G., Cox, R.H., Hernandez, O., Cole, R.J., and Dorner, J.W.: Orlandin: A non-toxic fungal metabolite with plant growth-inhibiting properties. *J. Agric. Food Chem.* (in press).

Dorner, J.W., Cole, R.J., Springer, J.P., Cox, R.H., Cutler, H., and Wicklow, D.: Isolation and identification of two new biologically active norditerpene dilactones. *Phytochem.* (in press).

Springer, J.P., Dorner, J.W., Cole, R.J., and Cox, R.H.: Terretoxin, a toxigenic compound from *Aspergillus Terreus*. *J. Org. Chem.* (in press).

Cutler, H.G., Crumley, F.C., Cox, R.H., Cole, R.J., Dorner, J.W., and Latterell, F.M.: Turrialbin: A new toxin from *Diplodia macrospora*. *J. Agric. Food Chem.* (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30061-02 ECB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Chemical Characterization of Potentially Complex Toxicological Mixtures. Yusho Oil and Used Transformer Fluids.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. D. McKinney	Supervisory Research Chemist	ECB NIEHS
OTHER:	P. A. Albro	Research Chemist	ECB NIEHS
	J. R. Hass	Research Chemist	ECB NIEHS
	E. E. McConnell	Veterinary Pathologist	EBB NIEHS

COOPERATING UNITS (if any)

Comparative Biology Section

LAB/BRANCH
Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.3	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

More extensive examination of new and used PCB-based transformer fluids indicate the presence of at least three classes of chlorinated aromatic compounds (chlorinated benzenes, biphenyls and diphenylethers) in the used fluid. A general scheme for future analysis of such fluid is emerging which uses a combination of UV-monitoring, chromatography and perchlorination techniques. Such techniques should enable class separation of such compounds in complex mixtures.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: A variety of chromatographic and spectroscopic methods have been used to isolate and identify fractions/components of these complex mixtures including gas, gel permeation, high pressure liquid and other column chromatography and ultraviolet, infrared and nuclear magnetic resonance, and mass spectrometry. Neutron activation analysis was also used.

MAJOR FINDINGS AND PROPOSED COURSE: Further study and analysis of Yusho oil has been suspended pending the availability of a new batch of oil to repeat the study and provide definitive data for publication purposes.

More extensive examinations of new and used PCB-based transformer fluids have been carried out since the preliminary work. The major finding was the presence of relatively large amounts (8 mol. or 11 wt percent) of chlorinated diphenylethers in the used fluid. Since the fluid contains major amounts of at least three classes of chlorinated aromatic compounds, it has presented difficult problems in separation science. For example, spiking with radiolabeled chlorinated dibenzofurans was required in isolating the furan fraction in the fluid. A general scheme for future analysis of such fluids is emerging which utilizes a combination of UV-monitoring, chromatography and perchlorination techniques to avoid arbitrary "cuts" and solvent changes, etc. Such techniques should enable class separation of such compounds in complex mixtures.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: PCBs are widely dispersed contaminants of the environment partly as a result of their industrial use as transformer and heat exchange fluids. In attempting to understand their environmental fate and distribution and associated toxicological impact, it is of considerable interest to determine the nature and extent of the alterations and rearrangements of such PCB-based fluids after extended use at high temperatures. Such thermodynamically derived PCB mixtures appear to present considerably different toxicological problems as evidenced by the "Yusho disease" of Japan.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30062-02 ECB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Immunochemistry of Dioxin Action on the Lymphocyte and Other Halogenated Aromatic Hydrocarbons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. I. Luster	Senior Staff Fellow	ECB NIEHS
OTHER:	G. A. Boorman	Veterinary Pathologist	EBB NIEHS
	K. Chae	Chemist	ECB NIEHS
	P. W. Albro	Research Chemist	ECB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.2	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of lymphocyte function following brief in vitro exposure of mouse spleens to 2,3,7,8-tetrachlorodibenzodioxin were reported last year. Further studies have shown no morphological effects by this exposure via EM. Autoradiography studies were not successful. Preliminary studies have indicated that the mitogenic suppression can occur directly in vivo by splenic perfusion (via the splenic artery) with TCDD/DMSO/culture media complexes. TCDD uptake was shown to be almost complete using ^{14}C-TCDD. Other studies involving pre/postnatal exposure of TCDD in $B_6C_3F_1$ mice have suggested that the immune suppression may be due to lymphotoxicity at higher TCDD levels and a defect in T-cell maturation at lower levels. B-cells and macrophages are not involved and none of the cell types produce suppressor activity. 2,3,7,8-tetrachlorodibenzofuran appears to act under similar mechanisms (Project # Z01 ES 30071-01 EBCB).

PROJECT DESCRIPTION

METHODS EMPLOYED: Mitogen studies have been adapted in co-cultures with purified B, T or macrophages. The techniques are currently employed in cancer patients.

MAJOR FINDINGS AND PROPOSED COURSE: TCDD may bind directly to a lymphoid cell, inducing lymphotoxicity and eventual loss of normal immune function (cell mediated). This cell is presumably a T-cell. Less clear, at lower concentrations, TCDD is not lymphotoxic but does effect T-cells. This is not through suppressor activity but probably through a defect in maturation. Attempts are being made to elucidate the maturational defect. Studies have been initiated to detect and isolate TCDD receptors on lymphocytes and other cell types.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: TCDD is an environmental contaminant. Since it effects the immune system of laboratory animals, it is suspect of causing similar alterations in exposed humans. Studies of the mechanisms of the immune effects may lead to a possible cure.

PUBLICATIONS

Luster, M.I., Clark, G., and Lawson, L.D.: Effect of brief *in vitro* exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on mouse lymphocytes. *J. Environ. Path. Toxicol.* (in press).

Faith, R.E., and Luster, M.I.: Investigations on the effects of TCDD on parameters of various immune functions. *N.Y. Acad. Sci.* (in press).

Luster, M.I., Faith, R.E., and Clark, G.: Laboratory studies on the immune effects of halogenated aromatics. *N.Y. Acad. Sci.* (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30063-02 ECB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Molecular Orbital Calculations on Molecules of Environmental Interest

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. E. Parker	Research Chemist	ECB NIEHS
OTHER:	J. R. Hass	Research Chemist	ECB NIEHS

COOPERATING UNITS (if any)
M.M. Bursey, Dept. of Chemistry, University of North Carolina, Chapel Hill, NC

LAB/BRANCH
Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Molecular orbital calculations have been done to determine minimum energy configurations of neutral molecules and positive and negative ions with respect to the mass spectral analysis of compounds of environmental interest.

PROJECT DESCRIPTION

METHODS EMPLOYED: Programs were obtained from the Quantum Chemistry Program Exchange. These programs were modified as needed and run on the IBM 360/70 at the Triangle Universities Computation Center.

MAJOR FINDINGS AND PROPOSED COURSE: The INDO/CNDO/2 and MINDO/3 methods have been tried on the molecules of interest, after the programs were modified to handle the large number of atoms and the types of atoms needed for the study. The structures of negative as well as positive ions were studied using both molecular orbital methods. For certain halogenated compounds, non-convergence was found which seems to correspond to ionization to a dissociative state. For systems with a single aromatic ring, minimum energy geometries and relative energies predicted by these two programs seem to agree. Different results were obtained for two-ring systems, such as biphenyls, where preliminary results seemed to indicate that the INDO/CNDO/2 method may give invalid results due to the overestimate of non-bonding interactions. This project will be continued by investigating the use of these programs for other molecules of environmental interest.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Mass spectrometry is widely used for the identification and analysis of compounds of environmental interest. Mass spectrometry allows one to deduce structure through spectral interpretation, even where no reference compound is available. Correct interpretation of the data depends on the understanding of the gaseous ion chemistry involved. Molecular orbital theory allows the calculation of minimum energy configurations and charge distributions which are required for a complete understanding of fragmentation reaction pathways.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30064-02 ECB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Field Desorption Mass Spectrometry Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. R. Hass	Research Chemist	ECB NIEHS
OTHER:	M. J. Bobenrieth	Visiting Fellow	ECB NIEHS

COOPERATING UNITS (if any)

R. Shapiro, Dept. of Chemistry, New York University
M.M. Bursey, Dept. of Chemistry, University of North Carolina

LAB/BRANCH
Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.3	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Adducts of phosphodiesters and polynucleotides with N-acetoxy-2-acetamidofluorene were prepared for analysis by field desorption mass spectrometry. Similar adducts of 9-bromomethylbenz(a)anthracene and benz(a)pyrene epoxydihydrodiol will be examined as well. Samples of selected dinucleotides were obtained.

PROJECT DESCRIPTION

METHODS EMPLOYED: Field desorption mass spectrometry, high performance liquid chromatography.

MAJOR FINDINGS AND METHODS EMPLOYED: The major adduct products from reaction of N-acetoxy-2-acetamidofluorene (NA-AAF) with phosphodinucleotides and small polynucleotides in citrate buffer were separated and isolated. As soon as the field desorption capability of the ZAB-2F mass spectrometer is routinely operational, these adducts will be used as models to find appropriate conditions for obtaining the field desorption mass spectra of small polynucleotide-NA-AAF adducts. Four adducts of 9-bromomethylbenz(a) anthracene with nucleosides have been obtained from Professor Robert Shapiro (New York University) and phosphodinucleotide adducts of benzo(a)pyrene epoxydiol will be prepared following successful analysis of the NA-AAF adducts. Work, as yet unsuccessful, was undertaken to prepare useful emitters in-house.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Finding mass spectrometric methods for the analysis of small polynucleotide-carcinogen adducts will permit a rapid method for identification of these compounds, permitting studies of the effects of neighboring groups upon the reactivity of the site attacked. Such studies should illuminate the micro-environments important in environmental chemical carcinogenesis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30065-02 ECB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Studies of Mass Spectral Reactions in Field-Free Regions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. R. Hass	Research Chemist	ECB NIEHS
OTHER:	L. A. Levy	Research Chemist	ECB NIEHS
	M. J. Bobenrieth	Visiting Fellow	ECB NIEHS

COOPERATING UNITS (if any)

M.M. Bursey, Dept. of Chemistry, University of North Carolina, Chapel Hill, NC.

LAB/BRANCH
Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mass-analyzed ion kinetic energy spectra of alkoxide and carboxylate ions were obtained after collisional excitation. The positive ions detected implied the formation of unusual oxenium and acyloxy ions, respectively. The collision-induced dissociations of the M+1 ions resulting from chemical ionization of aliphatic esters were studied to gain knowledge concerning the fragmentation of even-electron ions. Computer programs have been developed in cooperation with Finnigan Corporation which permit data system acquisition and processing of ion kinetic energy spectra.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: High resolution mass spectrometry with metastable scanning.

MAJOR FINDINGS AND PROPOSED COURSE: Mass analyzed ion kinetic energy (MIKE) spectrometry is relatively new. As a result, there is no library of data available for comparison of spectra; it is important to collect such spectra since it has been proposed that ion kinetic energy spectra of ions will complement gas chromatography/mass spectrometry, at least to some extent. To this end we established fragmentation patterns for the analysis of ester ions produced in chemical ionization conditions and examined the behavior of organic anions undergoing charge reversal in the mass spectrometer on collision with small gas molecules. Alkoxide and carboxylate ions have been examined and found to give spectra easily correlated with structure.

Computer programs have been written to our specifications which enable the main beam to be selected under computer control, MIKE spectra or linked magnetic-electric sector scans to be acquired, peak profiles measured, kinetic energy release calculated and the horizontal scale to be marked in any combination of daughter ion mass or kinetic energy and/or neutral loss from the main beam. This software is still under development.

An interesting correlation between kinetic energy release for a particular type of chlorine loss from polychlorinated biphenyls and infrared stretching frequency of the C-Cl bond was noted. If this type of relationship is found to be general, information similar to that obtained by infrared spectroscopy will be available with the sensitivity and separation advantages of GC/MS added.

This project will be continued by completing the above experiments. In addition, these techniques will be extended to other environmental chemicals.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Mass spectrometry (in combination with gas chromatography) is the most sensitive and specific analytical method presently available for dealing with complex samples of environmental/biological origins. The results of this project will increase our understanding of the fundamentals of the technique as well as provide more specific information for structural analysis.

PUBLICATIONS

Bursey, M.M., Harvan, D.J., Parker, C.E., Pedersen, L.G. and Hass, J.R.: Consequences of charge reversal in gaseous formate and acetate ions: Acyloxy ions. J. Am. Chem. Soc. (in press).

Bursey, M.M., Hass, J.R., Harvan, D.J. and Parker, C.E.: Consequences of charge reversal in gaseous alkoxide ions: Oxenium ions. J. Am. Chem. Soc. (in press).

Hass, J.R., Bursey, M.M., Levy, L.A. and Harvan, D.J.: Structural correlations with kinetic energy release during loss of chlorine from nineteen selected chlorinated biphenyls. Org. Mass Spectrom. (in press).

Hass, J.R., Harvan, D.J. and Bursey, M.M.: Mass analyzed ion kinetic energy spectra of the protonated molecular ions of aliphatic esters. Anal. Chim. Acta 105: 129-137, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30066-02 ECB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Development of Synthetic Methods for Polyhalogenated Aromatics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.D. McKinney	Supervisory Research Chemist	ECB NIEHS
OTHER:	A. Norstrom	Guest Worker	ECB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Pd(II)-acetate catalyzed ring closure of halogenated diphenyl ethers and benzophenones is being utilized to synthesize halogenated dibenzofurans and fluorenones. Such polyhalogenated aromatic compounds are of current toxicological interest as potential persistent environmental agents. Dibenzofurans synthesized include two hexas (1,2,4,6,8,9 and 2,3,4,6,7,8), one tetra (2,3,7,8), one tri (2,3,8) and one disubstituted (3,7). 2,3,7,8-Tetrachlorofluorenone was also synthesized.

PROJECT DESCRIPTION

METHODS EMPLOYED: Synthetic techniques, organometallic reagents, functional group transformations. Mass spectroscopy, other spectroscopic methods (IR, NMR), chromatography (column, glc, liquid).

MAJOR FINDINGS AND PROPOSED COURSE: Several chlorinated dibenzofurans have been synthesized from the appropriate diphenyl ethers previously synthesized. Dibenzofurans synthesized include two hexas (2,3,4,6,7,8- and 1,2,4,6,8,9), one tetra (2,3,7,8), one tri (2,3,8) and one disubstituted (2,7). A related compound 2,3,7,8-tetrachlorofluorenone was synthesized in low yield from 3,3',4,4'-tetrachlorobenzophenone ring closure. Attempted syntheses of hexabrominated naphthalenes and tetrabrominated biphenylenes failed but such syntheses were not exhaustively investigated. These syntheses were performed in a special glove box laboratory.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There is still a great need for simple synthetic routes to many polyhalogenated aromatics. This project will, hopefully, give simple synthetic routes to many toxicologically interesting polyhalogenated aromatics which are known to persist in the environment.

PUBLICATIONS

Norstrom, A., Chaudhary, S.K., Albro, P.W. and McKinney, J.D.: Synthesis of chlorinated dibenzofurans and chlorinated amino-dibenzofurans from the corresponding diphenyl ethers and nitro-diphenylethers. Chemosphere (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30067-02 ECB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Characterization of the Binding of Heavy Metals in Biological Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.H. Cox	Research Chemist	ECB NIEHS
OTHER:	None		

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

^{1}H, ^{13}C and metal nuclear magnetic resonance (NMR) spectroscopy is being used to investigate the specific binding character of certain heavy metals using purified biopolymers, their monomer and other isolated and definable biological systems as models. An attempt is being made to correlate binding propensity with toxic effects of the heavy metals. Early work indicates that ^{207}Pb NMR offers a useful probe into the binding of lead.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Multi nuclei nuclear magnetic resonance (NMR) spectroscopy using biological models for studying the interactions between heavy metals. Isolation and characterization of specific binding site(s) in body tissue and fluid.

MAJOR FINDINGS AND PROPOSED COURSE: There is an increasing body of evidence that the transport and accumulation of heavy metals in body tissue and fluids is related to the binding of the metals in biological systems. Studies to date have shown that ^{207}Pb NMR chemical shifts offers a very sensitive probe into the binding of lead. For example, preliminary studies show lead chemical shifts to be dependent on the oxidation state of lead, the coordination number of lead, and the nature of the ligands binding to lead. Studies with the thiol donors glutathione and cysteine show a large (1500 ppm) downfield shift of the lead resonance upon binding to the thiol group. Future studies will examine the chemical shift changes upon binding of lead to a variety of ligand types and with binding to various biological systems in an attempt to correlate binding propensity with toxicity.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There is increasing evidence that certain heavy metals produced as energy by-products may accumulate in body tissue and fluid and present serious health effects. Binding propensity of the heavy metals and toxicity and accumulation may be correlatable. An understanding of the specific molecular level interactions involved in binding may permit one to predict, prevent, or reverse them.

PUBLICATIONS

Cox, R.H.: Carbon-13 and lead-207 NMR studies of organolead compounds. J. Mag. Res. 33: 61, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30072-02 ECB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of Polybrominated Biphenyls. Immune Response in Rodents.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. I. Luster	Senior Staff Fellow	ECB NIEHS
OTHER:	G. A. Boorman	Veterinary Pathologist	EBB NIEHS
	J. A. Moore	Supervisory Veterinary Medical Officer	RRP NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of PBB on the immune response were further evaluated following low level chronic and pre/postnatal exposure in mice and rats. In chronic exposure studies, rats were found to be more immune suppressed than mice (a 10X). T-cell functions were more suppressed than B-cell functions, although B-cell functions were effected at the highest dosage group as indicated by a decrease in functional immune tests as well as decreased resistance to infection with Listeria monocytogenes. No gram negative endotoxin hypersensitivity was found. Pre/postnatal exposure failed to induce any immune alteration although an increase in the number of colony forming units in the spleen and increased endotoxin hypersensitivity were noted.

PROJECT DESCRIPTION

METHODS EMPLOYED: Methodology for these studies included *in vitro* lymphocyte stimulation, delayed hypersensitivity reactions, antibody quantitation, bone marrow tests, endotoxin hypersensitivity, resistance to *L. monocytogenes* infection and macrophage phagocytosis.

MAJOR FINDINGS AND PROPOSED COURSE: Low level chronic PBB exposure in rodents induces immunosuppression. These effects are only noted near toxic levels indicating that PBB is not a potent immunosuppressive chemical as previously felt. *In utero* exposure to PBB in mice has no adverse effects on the immune system. These studies have been terminated.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Studies in humans contaminated by PBB have suggested altered immune functions. Confirmation studies in laboratory animals were required to help substantiate these observations.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30087-01 ECB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Chronic Toxicity of Technical and Analytical Pentachlorophenol in Cattle. Chemical Analyses of Tissues.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.E. Parker	Research Chemist	ECB NIEHS
OTHER:	H.B. Matthews	Research Chemist	LP NIEHS
	E.E. McConnell	Comparative Pathologist	EBB NIEHS
	J.R. Hass	Research Chemist	ECB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.8	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Liver and adipose tissue from cows fed different proportions of analytical and technical grade pentachlorophenol were extracted and analyzed for chlorinated dibenzodioxins and dibenzofurans.

PROJECT DESCRIPTION

METHODS EMPLOYED: Gas chromatography/mass spectrometry/computer system.

MAJOR FINDINGS AND PROPOSED COURSE: Dioxin and furan levels in liver and adipose tissue increased with increasing amounts of technical versus analytical grade pentachlorophenol (PCP). The total amount of PCP fed was constant for all groups. Higher levels (by a factor of 10^2-10^3) of dioxins were found in liver than in adipose tissue, and the proportions of isomers that accumulated were different from the proportions in technical PCP. The same dioxin and furan isomers seemed to accumulate in both tissues, and these seemed to be the isomers with chlorine atoms in the 4 lateral positions. The relative proportions of different isomers and the ratio of dioxins to furans differed for the different tissues.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Possible dioxin contamination of dairy herds has been attributed to the exposure of cattle to PCP-treated lumber. Since the practice of housing cattle in barns constructed of treated lumber is widespread throughout the dairy and beef industry, this exposure may constitute a possible human health hazard. This study was part of a project to relate tissue levels and pathology to exposure.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30074-01 ECB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Development of Program in Bioinorganic Chemistry. Phase I - Literature Survey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. D. McKinney	Supervisory Research Chemist	ECB NIEHS
OTHER:	D. Boline	Visiting Researcher	ECB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Chemistry Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A program in bioinorganic chemistry is being initiated. This development will involve three stages, viz. (1) assessment of the state-of-the-art in inorganic residue analysis; (2) development of separation methodologies compatible with the selected analytical measurement techniques; (3) the application of validated methods to actual biological problems. This project encompasses stage 1.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

METHODS EMPLOYED: Standard methods and procedures used to search the scientific literature and prepare scientific reports.

MAJOR FINDINGS AND PROPOSED COURSE: A program in bioinorganic chemistry is being initiated. This development will involve three stages, viz. (1) assessment of the state-of-the-art in inorganic residue analysis; (2) development of separation methodologies compatible with the selected analytical measurement techniques; (3) the application of validated methods to actual biological problems. This project encompasses stage 1.

The initial phase would involve a great deal of literature searching, screening, etc. to assess the state-of-the-art in methods (both for speciation and elemental analysis), interaction with various Institute programs and scientists to find out what types of problems they are having in working with inorganics, e.g. heavy metals, assessment of the inorganics of greatest environmental concern, and interaction with the laboratories both private and academic to learn about their efforts in this area. The end product would be a document which compiles and interrelates this material and makes recommendations for future work. Phase I should be completed within this reporting period.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Relative to the organic residues in our environment, the inorganic residues have received little attention with regard to potential as human hazards. The environmental load of inorganics is expected to increase as a result of their being by-products of energy technologies. Development of a strong program in bioinorganic chemistry is essential to meeting this need.

SCIENCE APPLICATIONS, INC. - La Jolla, California 92038
(NIH-N01-ES-8-2105)

TITLE: Analysis of Total Organic Chlorine and Bromine Residues in Human Body Fluids and Tissues

CONTRACTOR'S PROJECT DIRECTOR: J. Reed

PROJECT OFFICER (NIEHS): J. D. McKinney, Ph.D., Supervisory Research Chemist, ECB

DATE CONTRACT INITIATED: December 20, 1977

CURRENT LEVEL (3 years): $169,712

PROJECT DESCRIPTION

OBJECTIVES: Analysis of 1500 to 2000 samples per year of breast milk, formula, blood serum, and tissue for total organic chlorine (TOCl) and bromine (TOBr) of whole fluid or wet tissue or for total soluble organic chlorine (TSOCl) and bromine (TSOBr) content of portions of extractable lipids. The desired detection thresholds range from 5-20 ng chlorine and 0.1-15 ng bromine/gm milk.

METHODS EMPLOYED: Somogyi method of desalting followed by standard methods of sample preparation for neutron activation analysis (NAA).

MAJOR FINDINGS AND PROPOSED COURSE: The contractor's major efforts to date have included completion of literature searches, performance of NAA on blanks, reagents, and solvents and development and validation of methodology for desalting human milk and serum. It was shown that the originally proposed precipitation desalting technique is not compatible with NAA measurement. A new method was developed based on Bio-Gel P-2 filtration which is compatible. Additional methodology will be developed for milk substitutes (formula) and placenta tissue extracts. Analyses of the first complete sets of mother's milk and blood are in progress.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Obtainment of accurate and reliable results that lead to meaningful interpretation of the transfer of PCB's and DDE from mother to child through placental tissue membranes or through breast milk requires the separation of inorganic bound chloride and bromine from inorganic chlorides and bromides prior to neutron activation analysis for TOCl, TOBr, TSOCl and TSOBr. This study integrates contract NIH-N01-ES-7-2141 to provide a mass balance which indicates whether all the halides are accounted for by the PCB's and DDE. In this manner, the results of the contract for the analysis of PCB's and DDE in human body fluids and tissues can be validated. This study will help resolve the important epidemiological effects of possible transplacental and breast milk transfer of environmental contaminants from mothers to babies in the United States.

WARF INSTITUTE, INC. - Madison, Wisconsin 60616
(NIH-N01-ES-7-2141)

TITLE: Analysis of PCB's and DDE in Human Body Fluids and Tissue

CONTRACTOR'S PROJECT DIRECTOR: D. L. Hughes

PROJECT OFFICER (NIEHS): J. D. McKinney, Ph.D., Supervisory Research Chemist, ECB

DATE CONTRACT INITIATED: September 30, 1977

CURRENT LEVEL (3 years): $267,656

PROJECT DESCRIPTION

OBJECTIVES: Analysis of 1000 to 1200 samples per year of breast milk, formula, blood serum and placental tissue for polychlorinated biphenyls (PCB's) and 1,1-bis(p-chlorobiphenyl)-2,2-dichloroethane (DDE). The desired detection thresholds range from 0.5 to 50 ppb depending on the type of sample.

METHODS EMPLOYED: Gas liquid electron capture chromatography and usual sample preparation, clean-up, extraction and lipid determination techniques.

MAJOR FINDINGS AND PROPOSED COURSE: This contract provides for development of the methodology for the analysis and the generation of suitable and reliable analytical data on PCB's and DDE in human body fluids and tissues, and is interfaced with contract (NIH-N01-ES-8-2105) for the analysis of total organic chlorine and bromine in same media. Contract NIH-N01-ES-8-2105 serves as a check and validation of the significance of the results. Method validation studies for human milk, blood (serum) and placenta tissue have essentially been completed. The contractor is in the process of setting up quality controls and will soon initiate analysis of the first sets of mother's samples. The contractor is serving as sample split point for the study. They will do first thaw and homogenization of sample, remove aliquots for other contractor and our purposes and retain and weigh in sample for their purposes. The first sets of mother's samples have been received by the contractor. Additional relevant experience has been gained by the contractor during this reporting period by analyzing a limited number of monkey samples from animals associated with PCB feeding studies.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The possible effect of transplacental and breast milk transfer of environmental contaminants from mothers to babies is an important and recurring epidemiological question. It has been shown that environmental contamination of breast milk occurs in the United States. Organohalogen pesticides and the polychlorinated biphenyls (PCB's) are widespread contaminants of breast milk. This study provides an integral part of an overall study of the possible widespread contamination of breast milk by environmental contaminants such as PCB's and their effects on infant development and health.

IIT RESEARCH INSTITUTE - Chicago, Illinois 60616
(NIH-NO1-ES-8-2115)

TITLE: Radiosynthesis of ^{14}C-2,3,7,8-Tetrachlorodibenzofuran (TCDF)

CONTRACTOR'S PROJECT DIRECTOR: Allan P. Gray, Ph.D.

PROJECT OFFICER (NIEHS): J. D. McKinney, Ph.D., Supervisory Research Chemist, ECB

DATE CONTRACT INITIATED: March 27, 1978

CURRENT LEVEL (150 days): $30,000

PROJECT DESCRIPTION

OBJECTIVES: Synthesis of 10 millicuries of ^{14}C-radiolabeled 2,3,7,8-TCDF at the highest specific activity reasonably achievable but not less than 50 millicuries/millimole and with a chemical and radiochemical purity greater than 98 percent.

METHODS EMPLOYED: Usual radiosynthetic techniques.

MAJOR FINDINGS AND PROPOSED COURSE: The compound, 2,3,7,8-TCDF has been previously synthesized unlabeled by IIT; however, its synthesis as a radiolabeled compound will require many different considerations and development of methodology. In this regard, two aborted attempts by the proposed contractor to prepare this compound labeled has shown that the selection of which precursor compound should contain the label and the reaction intermediates involved are critical in the radiosynthesis. This result was unexpected and appears to be related to the high specific activity and accompanying radiochemistry of "hot" free radical intermediates. Therefore, it was necessary to repeat the entire reaction scheme using an entirely different and more radiochemically reliable route, viz. via O-nitrochlorobenzene. The reaction proceeded smoothly and reproducibly when the radioactivity was split between the two coupling reactants. The desired 2,3,7,8-TCDF is currently being isolated from the crude reaction mixture of chlorinated furans formed in the final step.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The chlorinated dibenzofurans occur as contaminants in commercially produced polychlorinated biphenyl (PCBs). Currently there is little known about the toxicity and biological fate of these compounds. It is presently assumed that the 2,3,7,8-TCDF is the most toxic member of the class of chemicals. Studies at NIEHS have shown it to be highly toxic for the guinea pig, but markedly less toxic for mice and rats. Studies on these species' difference in regard to variation in adsorption, metabolism, distribution and excretion are urgently needed. Such studies are most rapidly and conveniently done using radiolabeled compounds.

RESEARCH SERVICES BRANCH

RESEARCH SERVICES BRANCH
Summary Statement

The Research Services Branch plans and coordinates new facilities for the Institute as well as operates and maintains existing facilities. It also provides a variety of research support functions such as laboratory instrumentation services, glassware and media preparation, arts, graphics, and photography.

Renovation of existing facilities has often lagged behind demand, principally due to a significant turnover in personnel and lack of new positions. The new facility currently under construction is also placing greater demand on the engineering personnel within the Research Services Branch as well as administrative personnel with the Office of the Associate Director.

Administration: Mr. Earl Cook was appointed Chief of the Branch and Mr. Lee Vaughn retired, thus creating vacancies in the top positions in both the design and facilities operation areas. An extensive search for qualified engineers resulted in the appointment of Mr. Nathan De Witt to head the design areas, and Mr. Warren Jones to head the facilities operation area. In anticipation of a necessary staff growth related to occupancy and operation of the new facility, we are proposing establishment of two Sections in RSB, the Engineering Design Section and Facilities Engineering Section. The latter section will house, in addition to the Shops Unit, the new Operations and Maintenance Unit, with major responsibility for the new facility, and ultimately, the greatest number of employees in the Branch.

A Research Services Branch Instruction Manual was developed and distributed to all RSB employees. The purpose of the Manual system is to provide a means for the Branch Chief, Section Chiefs and Unit Supervisors to publish and control instructions and information on policies and procedures to be used in administering the work of RSB. Also, a booklet to be titled "A Guide to Research Services Branch Services" has been written and will be issued to all NIEHS employees in the next few months.

There is a significant need for an administrative support position in the Office of the Branch Chief to assist in day-to-day management of the Branch, to improve administrative practices, and most important, to free the Chief for the critical coordination activities between the NIEHS, and the Project Engineer and Construction Manager for the new facility. These matters have been addressed inadequately until now because of a lack of manpower.

Facilities: NIEHS currently utilized 197,000 square feet of leased space. The principal interim site provides 154,000 square feet in a complex of 19 buildings. The institutes has a staff housing capability in excess of 400 permanent fulltime employees. Facilities operation, maintenance, and alterations are carried out by the facilities engineering staff. One of its major activities has been renovating laboratory and office space to meet specific program needs. An additional 7,000 net square feet of temporary office space is to be provided through the use of trailers that will be located on the permanent site. An animal building of approximately 5,000 net square feet has been leased to accommodate CMB animal breeding and production requirements.

New NIEHS laboratory, administrative and support facilities are being constructed on a 509 acre tract of land in the Research Triangle Park that is in close proximity to the current facilities. The new facilities, designed to NIEHS requirements, were funded by a $67 million Congressional appropriation. A construction management firm was selected in December 1976; construction contracts were subsequently awarded and initial construction begun in April 1977. Construction of the laboratory and administrative buildings will be completed and Institute staff are to begin occupying the new facilities in 1980. Prior to that time, during the summer of 1979, RSB staff will occupy the support facilities. NIEHS will continue to occupy the principal current facilities, while "off site" leased facilities will be relinquished.

The table below outlines the function and amount of space in both the current and new facilities. Staffing capability is projected at approximately 800 for the new facility and 400 in the existing quarters.

Program Facility	Current Leased Facilities (gross sq.ft.)	Permanent Facility (gross sq.ft.)
Laboratories (include 8097 sq. Ft. off site)..	69,155	129,640
Animal....................................	24,353	81,860
Biostatistical Labs........................	4,148	20,460
Direct Lab Support.........................	7,750	37,880
Office (includes 8800 sq. ft. off site).......	32,590	25,380
Conference Facilities & Public Space..........	2,335	7,180
Cafeteria..................................	2,090	12,070
Library....................................	3,650	0
Other......................................	0	19,530
Subtotal, Program Facility...	146,071	334,000
Support Services		
Power Plant & Incinerator....................	19,580	51,706
Shops......................................	3,994	29,883
Warehouse..................................	27,366	26,935
Communications & Electrical..................	0	9,749
Subtotal, Support Services..	50,940	118,273
Total......................................	197,011	452,273

Engineering Design: This group provides the architectural and engineering support required for planning new NIEHS facilities, improvements, major repairs, and alterations through in-house design, direct contact with A/E firms, or consultation, liaison and review functions for projects contract and administered by OES, NIH. The group also provides architectural and engineering support required for the administration and inspection of NIEHS construction provided under direct contract. This includes reviewing shop drawings and coordination input into the permanent site construction.

A continuing search is being made for qualified engineers to fill two mechanical and one electrical engineering vacancy. Numerous needed alterations, renovations, and repair projects are being held up because of lack of personnel to accomplish the in-house design. An engineering consultant has been appointed to provide design expertise in mechanical systems and ventilation. He will give initial attention to many critical ventilation problems throughout the Institute. Also, the Contracting Office is developing a contract for the professional services of an Architectural Engineering Consulting firm to design some of our major projects. In the meantime, NIH has permitted RSB to use its A/E contractor to develop plans and specification for two projects: (1) the renovations to Building 7, and (2) a new chemical and self-service store building to be constructed just north of Building 10.

During the year, major activities have been the administration and inspection of the Building 7 renovations; award of contracts for site work, utilities and installation of trailers on the permanent site; and coordination for the permanent site construction.

Facilities Engineering: This group provides engineering, craft and laboring services necessary for operating and maintaining NIEHS buildings, grounds, utility plant, systems, and related equipment; and contributes engineering evaluation of operational and maintenance aspects of new construction.

During the year, Mr. LeRoy Hood was appointed as Supervisor of the Shops Unit. A search is being made to select a supervisory engineer to head the new Operations and Maintenance Unit and to fill several support positions in this unit. The hiring of qualified personnel is critical with the need to provide training, and establish procedures for operating and maintaining the new NIEHS facilities.

Laboratory Services: Centralized glassware and media preparation services are provided to all laboratories. Glassware processing average in excess of 60,000 pieces per month; a number of types of disposable glassware are also supplied. Media preparation averages 1500-2500 liters per month of a wide variety of types, including tissue culture and microbiological preparations. During the year, because of increased demand for services, new personnel were hired and training completed to add a second shift to the media preparation unit. The second shift was begun but stopped when the new employees resigned. Turnover in personnel has temporarily prevented the re-establishment of the second shift operation.

Laboratory instrumentation services include design and fabrication of specialized instrumentation, fabrication of assorted laboratory paraphernalia, and repair and maintenance of laboratory equipment.

The unit, consisting of both the Machine and Electronics Shop, is completing 900 to 1000 work requests annually for the scientific staff plus many small repair work requests for RSB.

The Arts, Graphics and Photography Unit provides illustrations, graphics and photographic services to the Institute. During the past year, over 900 work orders were processed. Because of the increased demand for services, the turn-around time for both graphics and photography has increased to 10 working days. In addition, a contract has been awarded to a local photographer for photographic processing services.

GPO 860 892

Library
National Institutes of Health
Bethesda, Maryland 20014

LIBRARY
NIH
Amazing Research.
Amazing Help.
http://nihlibrary.nih.gov

10 Center Drive
Bethesda, MD 20892-1150
301-496-1080

DATE DUE

GAYLORD PRINTED IN U.S.A.

WERT
BOOKBINDING
MIDDLETOWN, PA
JULY '80
We're Quality Bound

NIH LIBRARY
4 0138 1497

NIH LIBRARY
3 1496 00181 8239